Genome-wide regional heritability mapping identifies a locus within the *TOX2* gene associated with Major Depressive Disorder

Short title: Regional Heritability Mapping for MDD

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Table number: 2. Figure number: 3. Supplementary file:1. Words count: Abstract:244; Main text: 3933

Abstract

Background: Major Depressive Disorder (MDD) is the second largest cause of global disease burden. It has an estimated heritability of 37% but published genome-wide association studies have so far identified few risk loci. Haplotype-block-based regional heritability mapping (HRHM) estimates the localized genetic variance explained by common variants within haplotype blocks, integrating the effects of multiple variants, and maybe more powerful for identifying MDD-associated genomic region.

Methods: We applied HRHM to GS:SFHS, a large family and population based Scottish cohort (N=19,896). Single-SNP and haplotype-based association tests were used to localize the association signal within the regions identified by HRHM. Functional prediction was used to investigate the effect of MDD-associated SNPs within the regions.

Results: A haplotype block across a 24kb region within the *TOX2* gene reached genome-wide significance in HRHM. Single-SNP and haplotype-based association tests demonstrated that five out of nine genotyped SNPs and two haplotypes within this block were significantly associated with MDD. The expression of *TOX2* and a brain-specific LncRNA RP1-269M15.3 in frontal cortex and Nucleus accumbens basal ganglia, respectively, were significantly regulated by MDD-associated SNPs within this region. Both the regional heritability and single SNP-associations within this block were replicated in the UK-Ireland group of the most recent release of the Psychiatric Genomics consortium (PGC2-MDD). The SNP-association was also replicated in a depressive symptom sample that shares some individuals with PGC2-MDD.

Conclusion: This study highlights the value of HRHM for MDD and provides an important target within *TOX2* for further functional studies.

Key Words: Regional heritability; HRHM; TOX2; MDD; haplotype block; Genome-wide analyses

Introduction

Major Depressive Disorder (MDD) is ranked as the second leading contributor to the global disease burden in terms of the years lived with disability(1). The narrow sense heritability of MDD has been estimated to be approximately 37% by twin studies(2), suggesting a substantial contribution from genetic factors. In efforts to identify specific genetic risk factors for MDD, family-based linkage studies have identified several significant peaks in certain families, but the findings have been inconsistent(3). GWAS studies of unrelated participants have successfully identified hundreds of loci associated with other psychiatric disorders(4), but for MDD, only four genome-wide significant and replicable loci have been identified by two large GWAS studies, one on a refined MDD phenotype for Chinese Women and one on self-report-based depression using less intensive phenotyping in a much larger European sample(5-7).

Several factors may be responsible for the comparatively sparse GWAS results in MDD. First, MDD is likely to have a highly polygenic genetic architecture where the disease risk is conferred by many causal variants of small effect(8,9). Combined with the high prevalence of MDD(10) and the possible incomplete LD between genotyped SNPs and causal SNPs, single-SNP based genome-wide association tests may have insufficient power to detect individual causal variants(11). Second, clinical heterogeneity has been shown in MDD between populations(6,12), and this may lead to difficulties in identifying causal variants across cohorts(13). Whilst GWAS sample sizes for MDD are increasing and efforts to refine MDD phenotype are in progress(5,7), alternative methodologies for detecting the signal arising from causal variants within and across families may also be productive.

Regional heritability mapping (RHM) is a method used to identify small genomic regions accounting for a significant proportion of the phenotypic variance in a trait of interest(14). In contrast to single-SNP based tests, RHM integrates effects from multiple SNPs by utilizing a regional genetic relationship matrix estimated from SNPs within a region. The matrix is constructed for each region defined by a sliding window across the genome, and is then used to estimate the variance explained by the variants within the region in a linear mixed model(14). The major advantage of RHM is that the regional genetic relationship matrices not only tag the effect of genotyped variants, but also measure the effect of un-genotyped and rare variants, including those associated with the SNPs but with individual effects too small to be detected by GWAS(14,15). Previous studies have shown that RHM has greater power to detect rare variants and multiple alleles in regions where GWASs provided null findings(15-17). In 2014, Shirali .,*et.al* developed a haplotype-block-based heritability mapping (HRHM) method as an improved version of RHM. HRHM utilizes haplotype blocks as the unit of mapping therefore the identified blocks have less complex local LD structures(18).

In this study, we applied HRHM to a homogenous sample of approximately 20K Scottish participants containing both closely- and distantly-related subjects with genome-wide genotyping data and a standardised structured clinical MDD diagnosis(19). We sought to identify genomic regions conferring risk for MDD, which were then further explored using single-SNP- and haplotype- based association tests. We then examined the functional effects of the MDD-associated SNPs within the identified block. Finally, replication analyses were performed in independent samples for both the regional heritability and SNP association results.

Methods and Materials

The Tayside Research Ethics Committee (reference 05/S1401/89) provided ethical approval for the study. Participants all gave written consent, after having an opportunity to discuss the project, and before any data or samples were collected.

Datasets

Discovery sample: Generation Scotland: The Scottish Family Health Study (GS:SFHS) contains 21,387 subjects (N_{male}=8,772, N_{female}=12,615; Age_{mean}=47.2(SD=15.1)), who were recruited from the registers of collaborating general practices in Glasgow, Tayside, Ayrshire, Arran and Northeast regions of Scotland. At least one first-degree relative aged 18 or over was required to be identified for each participant(19,20). A structured clinical interview was used for the diagnosis of lifetime DSM-IV mood disorders (SCID)(21,22). Details of MDD diagnosis, genotyping, quality control (QC) and imputation methods are described in Text s1. In total 561,125 genotyped and 8,642,105 post-imputation autosomal SNPs passed QC criteria were available for 19,896 participants (2,659 MDD cases and 17,237 controls) for subsequent analyses.

Replication sample 1: UK Biobank

Data used in this study were provided as part of UK Biobank project reference #4844. Details for genotyping, quality control, imputation and phenotyping are described in Text s2. In brief, genotyping data was available for 152,729 UK Biobank participants

recruited in United kingdom(23). The probable MDD phenotype was created based on the putative MDD definition established in Smith et al(2013) using responses to a touchscreen questionnaire (UK Biobank 2011b)(24), from self-report information, and from inpatient records via linkage to hospital episode data(Text s2). After quality control and removing subjects who were in both GS:SFHS and UK Biobank datasets, and one of each pair of close relatives (relatedness > 0.05) of GS:SFSHS participants or the remained UK Biobank participants, 1,198,327 SNPs for 24,015 subjects with putative MDD phenotype available (8,143 cases and 15,872 controls) remained in downstream analyses.

Replication sample2: PGC Major Depression Dataset (PGC2-MDD)

The Psychiatric Genomics Consortium provided individual genotypes (best guess) of imputed SNPs for participants from 22 cohorts in PGC2-MDD(Table s1). All cases met DSM-IV criteria for life MDD, the majority of them were ascertained clinically. Most control samples were screened and participants with lifetime MDD were removed(Table s1). Details for genotyping, quality control, imputation and phenotyping are described in Text s3. After quality control and removing subjects overlapped with GS:SFHS and UK Biobank dataset, 32,554 subjects of European ancestry (13,261 cases and 19,293 controls) were used in downstream analysis. Consistent with earlier work(25,26), we grouped the 22 cohorts into 7 groups based on the country of ancestor information for regional heritability analysis(Table s1).

Replication sample3: Depressive symptom datasets (DS) (this sample contains overlapping individuals with replication sample 1 and 2)

Okbay *et.al*(2016) carried out a GWAS meta-analysis (N = 180,866) on three samples using depressive symptoms as the trait of interest (27). The ascertained MDD diagnosis information was available for two samples (PGC1-MDD(N_{cases} = 9,240, $Nc_{ontrols}$ = 9,519) and the Resource for Genetic Epidemiology Research on Aging (GERA, N_{cases} = 7,231, $N_{controls}$ = 49,316))(27). For the third sample (UK Biobank (N = 105,739)), a continuous phenotype measuring the severity of depressive symptom had been created and used in the meta-analysis(27). Whilst this sample overlapped with the PGC2-MDD and UK Biobank samples, it provided results based upon a non-diagnostic quantitative measure of depressive symptoms and involved another large cohort, GERA(27).

Genome-wide haplotype-block-based Regional Heritability Mapping (HRHM)

Regional heritability mapping (RHM) is a method for detecting localized genomic regions where genetic variants contribute significantly to the variation of phenotype of interest(14). As an improved version of RHM, HRHM divides the genome into haplotype-blocks, based on the recombination hotspots in the genome(18). Details of HRHM are described in Text s4. In brief, in GS:SFHS the genotyped SNPs were mapped to 49,637 haplotype-blocks across the genome and the regional heritability was estimated and tested for each of the haplotype-blocks. A standard 'two-GRM' model incorporates two genomic relationship matrices (GRM); a regional genomic relationship matrix (rGRM) estimated from SNPs in the haplotype block and a

complement genomic relationship matrix (cGRM) estimated from all SNPs that are not included in in the haplotype block. These GRMs were jointly fitted as random effects in LMM. Covariates fitted as fixed effects include age, age^2, sex, 20 principal components. A Log likelihood ratio test (LRT) is applied to test the significance of random effect represented in rGRM by comparing a model with both cGRM and an rGRM fitted against a model including the cGRM but without an rGRM fitted. The genome-wide significance threshold for P values from LRT is 1.01×10^{-6}($N_{Bonferroni}$=49,637). This two-GRM model, while providing an unbiased estimate of regional heritability, was highly computationally demanding. To improve the calculation efficiency, a pre-adjustment strategy was applied in the genome-wide HRHM (Text s4). For haplotype-blocks that exceeded the genome-wide significant threshold, we re-tested the block using the two-GRM model to provide an accurate estimation of regional heritability in the target block. All the analyses were performed in REACTA(14,28). According to the GCTA-GREML Power Calculator, this study is well-powered for the GREML-based SNP heritability analysis (99.88%)(29).

Localized association tests for the significant haplotype block identified by HRHM in GS:SFHS

HRHM identified a significant block chr20:42555671-42579473, we performed a series of association tests to localize the association signals within this block in GS:SFHS.

1) Single-SNP-based association test for common SNPs within the identified haplotype-block.

Association tests were performed on genotyped and imputed common SNPs located in the significant haplotype-block chr20:42555671-42579473 using GCTA-MLMA (linear mixed model based association analysis)(30). The SNP effect was tested as a fixed effect, other covariates included age, age^2, sex and 20 PCs. To prevent the estimates of SNP effects from being confounded by the polygenic component and family structure, cGRM and cGRM$_{kin}$ were fitted simultaneously as random effects in the model(31). cGRM (complement-snp-set GRM) was the genomic relationship created matrix using all of the genotyped SNPs excluding the SNPs in the hit block; cGRM$_{kin}$ was the kinship relationship matrix (representing pedigree-associated genetic variation). cGRM$_{kin}$ was created by setting elements in cGRM that were less than or equal to 0.05 to 0(31). The estimated fixed effect (on the linear scale) was transformed to logit and liability scale using Taylor series approximation(32). Bonferroni multiple-testing-correction was performed for the P values for each SNP.

2) Single-haplotype-based association test

Single-haplotype-based association tests were performed for the common haplotypes (Frequency≥0.01) derived from the nine genotyped common SNPs located in the significant haplotype-block chr20:42555671-42579473 using GCTA-MLMA(30) for the full dataset and an unrelated dataset, and using famLBL(33) for a subset consisting of case-parent trios in GS:SFHS. Details of single-haplotype-based association test are described in Text s5.

Functional effects of MDD-associated-SNPs in the significant block

The significant haplotype-block chr20:42555671-42579473 is located in the intron region and a proportion of an adjacent exon of gene *TOX2*. To investigate the potential functional effects from variants within this block, we imputed the 9 genotyped SNPs within this block to 53 common SNPs based on HRC reference, all of them are non-coding SNPs. We performed single-SNP-based association test for each of them with MDD using GCTA-MLMA (the same method for genotyped SNPs). This identified 38 imputed SNPs significantly associated with MDD. We then examined the functional role of the 38 SNPs using the following functional annotation tools and analyses: the potential to affect the binding of transcription factors in Regulomedb(34), Genome Wide Annotation of VAriants(GWAVA), Genomic Evolutionary Rate Profiling (GERP)(35), brain-tissue-specific allelic effect on gene-expression(eQTL analysis) based on GTEX and BRAINEAC, and brain-tissue-specific allelic effect on DNA methylation in CpG loci(meQTL analysis). Details of these tools and analyses are described in Text s6.

Replication analysis

Regional heritability in the significant block identified in GS:SFHS

Individual genotypes in UKbiobank and PGC2-MDD(22 cohorts) were used to estimate the regional heritability of the target haplotype block in the two samples. The two-GRM model (rGRM+cGRM) was applied to provide accurate estimates. For PGC2-MDD, the regional heritability was estimated for each of the 7 groups defined based on country of ancestor (Table s1) as well as for the combined dataset.

Single-SNP based association test for the five significant SNPs(genotyped) within the significant block identified in GS:SFHS

For UK Biobank the single-SNP-based association tests were performed using a logistic model in PLINK(36). Covariates included age, sex, centre, batch and 15 principal components provided by UK Biobank. For PGC2-MDD the association test was performed using a logistic model for each individual cohort. Covariates include sex and 20 principal components (the age variable was not yet available for the full dataset at the time of this study). Meta-analysis was performed across all cohorts in each group to generate group-level association statistics. The meta-analysis was performed using the 'metagen' function in R package 'meta'. For the DS sample the GWAS summary statistics were downloaded from the website of social science GWAS consortium (http://www.thessgac.org/#!data/kuzq8).

Results

Genome-wide Haplotype-block-based regional Heritability Mapping (HRHM) was carried out for 49,637 haplotype-blocks using 56,1125 genotyped common SNPs in GS:SFHS for MDD (N_{case}=2,659, $N_{control}$=17,237). The regional heritability from each haplotype-block was tested using a pre-adjusted-GRM strategy in the linear mixed model. The Manhattan plot and qqplot for the LRT are shown in Figure 1. One haplotype-block covering a 24kb region in the intron region and a proportion of an adjacent exon of gene *TOX2* exceeded the genome-wide significant threshold ($P_{Bonf\_threshold}$=1.01x10^{-6}): hg19:chromosome20:42555671-42579473 (P_{lrt}=8.86x10^{-7}) (Figure 1). The two-GRM model confirmed the significance of this haplotype-block

(P_{lrt}=5.6 x10^{-7}) and the regional heritability (h_g^2) was estimated to be 0.008(0.006). The regional heritability of this block was more significant in females MDD (h_g^2=0.009, se=0.007, P_{lrt}=5.64x10^{-5}, N_{case}=1,893, $N_{control}$=9,818) than in males MDD (h_g^2 =0.003, se=0.004, P_{lrt}=0.02, N_{case}=765, $N_{control}$=7,420).

We further performed a series of association tests to disentangle the signal detected by HRHM in the significant block. Using the single-SNP-based association test, five of the nine genotyped common SNPs within the hit block were significantly associated with MDD (Table 1, s2). The five significant SNPs were in high linkage disequilibrium (LD) with each other (Figure 1D) and their minor alleles showed a consistent negative effect on the risk of MDD with the odds ratio ranging from 0.785 to 0.833(Table 1). Haplotype-based association tests for haplotypes derived from the nine SNPs showed that two out of the seven common haplotypes (frequency ≥ 0.01) were associated with MDD. One of these haplotypes contains the minor (protective) alleles of the five single-SNP-level significant SNPs and one contains the major (risk) alleles. The size and the direction of the effects of the two haplotypes were consistent with that estimated from the single-SNP based tests (odds ratio: 0.792 for the protective haplotype and 1.232 for the risk haplotype) (Table 2). Additional association tests on sub datasets (unrelated and case-parents-trio) showed that the risk haplotype was significantly associated with MDD in the unrelated dataset (Table s3), whereas the protective haplotype was significant in case-parents-trio dataset (Table s4).

The significant block overlapped with an enhancer active in multi-tissues and cell lines including astrocytes (Figure 2A)(37), and multiple alternative transcription start sites (TSSs) including a TSS primarily expressed in thalamus (the TSS labeled as 'p3@TOX2' in Figure 2A)(37), suggesting a potential regulatory role. To link the association signal from single variants with the potentially functional effects of those variants on disease-relevant biological processes, we identified 38 imputed SNPs in the target block significantly associated with MDD (Table s5), and predicted their potentially regulatory function using multiple predictors and statistics of non-coding DNA function, including the likelihood of affecting transcription factor binding, multi-genome-wide properties, evolutionary conservation, the *cis*-effect on gene expression of genes within a distance of 1MB and on DNA methylation. Among the 38 SNPs, two of them were annotated to be 'likely to affect TF binding' (score=2b) by regulomeDB, five obtained a GWAVA-TSS score ≥ 0.5 (suggesting 'functional') and five obtained a GERP-score >2 (suggesting 'constrained') (Table s6). Tissue-specific snp-*cis*-gene-expression (*cis*-eQTL) analyses were performed for the 38 SNPs using 11 brain tissues from GTEX and 10 brain tissues from BRAINEAC. The results from GTEX showed that the genotype of 30 of the 38 SNPs significantly stratify the expression of gene RP1-269M15.3 (LncRNA) in the tissue 'Nucleus accumbens basal ganglia' with the minor alleles significantly up-regulating the RNA expression level (Table s7)(Figure 2B). The results from BRAINEAC suggested that all of the 38 SNPs significantly stratify the expression of gene *TOX2* in frontal cortex (minor allele induces up-regulation) (Figure 2C) and gene C20orf62 (LncRNA) (minor allele induces down-regulation) in Cerebellar cortex (Table s8,s9). The results from meQTL analysis suggested that 30 of the 38 SNPs are significant meQTL SNPs in frontal cortex, and that particularly 19 of them significantly stratify DNA methylation of a

CpG locus cg24403644 (minor allele induces hypo-methylation) (Table s10). cg24403644 is located in a cluster of TSSs in *TOX2* (Figure 2) and shows differential methylation between human fetal and postnatal lifetime in frontal cortex and during the fetal brain development(38,39). Among significant SNPs in the *cis*-eQTL and *cis*-meQTL analyses, rs79645278 located in the peak of active enhancer (in astrocytes and other cell lines), and was predicted to be 'likely to affect TF binding' (2b) in RegulomeDB, having a GWAVA-TSS score of 0.5 and a GERP-score of 2.31(Figure 2A,B,C, Table s6).

The regional heritability detected in the hit block was replicated in the UK-Ireland group in PGC2-MDD with nominal significance (P_{lrt}=0.049, h_g^2=0.001,se=0.001), whilst it was not significant in other groups in PGC2-MDD and UK Biobank (Table s11). The single-SNP-based association test for the five significant SNPs (genotyped) in this block identified in GS:SFHS showed that all five were replicated in the DS sample; all five were also replicated in the UK-Ireland group in PGC2-MDD (Table 1. Results for individual cohorts were shown in Table s12 and Figure s1), but not in other PGC2-MDD groups or in the meta-analysed combined PGC2-MDD sample (Table s13); none of the five SNPs were replicated in the UK Biobank sample but all showed the same consistent direction of effect with that reported in the discovery sample (Table 1, Figure s1). Meta-analysis using all independent UK-Ireland replication samples (UK Biobank + 4 cohorts in PGC2-MDD-UK_Ireland) showed that all of the five SNPs reached nominal significance (Table s13, consistent sign with GS:SFHS as shown in Figure 3, using SNP rs6093898 as an example.

Discussion

The current study used a combination of genome-wide haplotype-block-based regional heritability mapping (HRHM), localized association tests and functional prediction to identify candidate genomic region associated with MDD. Using a large Scottish cohort GS:SFHS, a genome-wide significant haplotype block located in gene *TOX2* was identified by HRHM as a risk region for MDD. Association tests using both single SNPs and haplotypes within this block highlighted candidate contributing genetic variants for MDD. Replication analyses showed that the regional heritability in this block was nominally significant in the UK-Ireland groups in PGC2-MDD. The SNP-level association signals within the hit block were replicated in the UK-Ireland group in PGC2-MDD and a study of depressive symptom (DS) which has overlapping subjects from PGC2-MDD and UK Biobank.

As shown in this study, compared with single-SNP-based genome-wide association methods (GWAS), HRHM provided following advantages: (1) a smaller number of tests were performed therefore a less stringent threshold of genome-wide significance was applied. (2) Haplotype blocks rather than single SNPs were the unit of mapping, these are therefore relatively less dependent on the density of the genotype arrays and do not require the same SNPs to be typed or imputed in replication study. (3) HRHM applied a linear mixed model accounting for both polygenic component and family structure, and can be applied to both population and family data. (4) Since haplotype blocks were used as the unit of mapping, the identified locus has a less complex LD structure (Figure 1D), which will benefit the downstream identification of candidate variants.

306

307 To date, published GWASs have mapped associated variants to very few genes for
308 MDD (*LHPP, SIRT1, TMEM161B–MEF2C* and *NEGR1*)(5,7). In this study, the
309 identified haplotype block was located in gene *TOX2* (TOX high mobility group box
310 family member 2, also known as *GCX1*), indicating a new candidate gene for MDD.
311 *TOX2* is a putative transcriptional activator involved in the hypothalamo-pituitary-
312 gonadal system(40), and is located in a large genomic region which has been
313 previously reported as associated with depression symptoms in psychotic
314 illness(41,42). The same locus has also been weakly-associated with conduct disorder
315 in a previous study(43). Using available databases, we found that convergent evidence
316 from TSS by Fantom5 annotation (Figure 2A), histone modification markers and
317 Dnase peaks representing active enhancers by ENCODE annotation (Figure 2A), and
318 transcription factor binding prediction by RegulomeDB (Table s6) suggested a
319 regulatory function of this block. To test for the potential effects of the variants within
320 the block on gene expression, we performed brain-tissue specific *cis*-QTL analysis for
321 SNPs significantly associated with MDD within the block. The expression of a
322 LncRNA RP1-269M15.3 was significantly up-regulated by the minor alleles (minor
323 alleles are protective to MDD as shown in Table 1, s5) of candidate SNPs within the
324 block in Nucleus Accumbens, a tissue having been previously implicated in MDD(44).
325 RP1-269M15.3 was a multi-exon LncRNA with a multi-species conserved region
326 (Figure s2A) and was only expressed specifically in brain tissues (Figure s2B) and
327 therefore of potential function in brain tissues. Similarly, the expression of gene *TOX2*
328 was significantly up-regulated by the minor alleles of candidate SNPs in frontal
329 cortex, a relevant tissue of MDD as well (45). The regulatory effect of MDD-
330 associated SNPs in gene *TOX2* in frontal cortex is further supported by the meQTL

331 analysis on the same tissue. Combined with the fact that all of the 19 SNPs are both
332 meQTL and eQTL SNPs for gene *TOX2* in frontal cortex and the fact that hypo-
333 methylation has been previously suggested to be correlated with up-regulation of gene
334 expression(46), consistent evidence from both methylation and gene expression data
335 indicated that the minor alleles (protective) of MDD-associated SNPs up-regulate the
336 gene expression of *TOX2* in frontal cortex(Table s8,s10). Interestingly, the brain-
337 specific expression of both RP1-269M15.3 and *TOX2* were highly correlated ($r \geq 0.7$)
338 with a number of depression-related genes (for example, *LRFN5*, *GRM7*, *CRH*)(47,48)
339 in brain development (http://brainspan.org) (Table s14, s15), suggesting that the
340 expression networks involving those genes were potential targets of the effects from
341 candidate variants. These results are consistent with a previous study suggesting an
342 overrepresentation of MDD GWAS significant loci in CNS expression and the
343 regulation of gene expression in CNS during development(7).

344

345 The regional heritability in the identified block was nominally significant only in the
346 UK-Ireland group of PGC2-MDD. The five significant genotyped SNPs within the
347 block identified in GS:SFHS were replicated in DS sample and in the UK-Ireland
348 group in PGC2-MDD. UK Biobank sample failed to replicate any of them, although
349 they showed consistent sign of effect. Those results are likely attributable to the
350 phenotyping differences (diagnosed MDD in GS:SFHS, mostly diagnosed MDD in
351 PGC(49), putative MDD in UK Biobank and depressive symptom in DS), and the
352 clinical heterogeneity within MDD across PGC2-MDD groups as shown in Table
353 s10)(12). Notably, UK-Ireland which shows the most consistent replication results are
354 from the same country/region with GS:SFHS, so its cohorts are likely to have a

similar local genomic recombination pattern and LD structure with GS:SFHS and potentially carry alleles not common in other European cohorts, which may explain the better replication result from this group (Figure 3,s1).

There are, however, several limitations in the current study. Firstly, the re-adjustment strategy applied to genome-wide HRHM, whilst it reduced the computational burden, it was potentially excessively conservative in reporting true associations (observed LRT statistics were depleted from expectation, as shown in Figure 1D), which consequently reduced the power of HRHM(50). Secondly, phenotypic difference among discovery and replication samples impeded the complete replication of findings across all samples. UK Biobank samples are also from the same country/region as GS:SFHS, as are the UK-Ireland group of PGC2-MDD, but currently UK Biobank only have putative MDD information available for a small subset of genotyped participants. Ongoing clinical assessment of MDD and the genotyping work on this sample will potentially provide more power to the replication analysis for our findings in future data releases.

Conclusion

The present study showed the first application of genome-wide HRHM to a psychiatric disorder. A genome-wide significant region was identified by HRHM and the contributing genetic effect was localized to variants and haplotypes within the block. The results were partly replicated in two independent samples. Functional prediction and *cis*-eQTL analyses suggested that the genotype of associated-variants

within the block stratified the gene expression of a potentially functional LncRNA RP1-269M15.3 and gene *TOX2* in MDD-relevant brain tissues, which should be explored in further studies.

Data Access:

GS:SFHS data is available to researchers on application to the Generation Scotland Access Committee (access: http://generationscotland.org). The managed access process ensures that approval is granted only to research which comes under the terms of participant consent.

Financial Disclosures

AMMcI has previously received grant support from Pfizer, Lilly and Janssen. These studies are not connected to the current investigation**.** The author YNZ acknowledge the support from China Scholarship Council. The authors TKC and AMMcI acknowledge with gratitude the financial support received for this work from the Dr Mortimer and Theresa Sackler Foundation. PAT, DJP, IJD and AMMcI are members of The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (MR/K026992/1). Funding from the Biotechnology and Biological Sciences Research Council (BBSRC) and Medical Research Council (MRC) is gratefully acknowledged. DJM is an NRS Fellow, funded by the CSO. PN and CSH acknowledge support from the MRC. Other authors have no conflicts of interest.

Acknowledgements

We are grateful to the families who took part in GS:SFHS, the GPs and Scottish School of Primary Care for their help in recruiting them, and the whole GS team, which includes academic researchers, clinic staff, laboratory technicians, clerical workers, IT staff, statisticians and research managers. This work is supported by the Wellcome Trust through a Strategic Award, reference 104036/Z/14/Z. The Chief Scientist Office of the Scottish Government and the Scottish Funding Council provided core support for Generation Scotland. GS:SFHS was funded by a grant from the Scottish Government Health Department, Chief Scientist Office, number CZD/16/6.

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Legends

Table 1. Single-SNP-based association test results for five MDD-associated SNPs in discovery and replication samples.

Table 2 Haplotype-based association test results for common haplotypes derived from the nine genotyped common SNPs in GS:SFHS.

448 Adjusted P: Bonferroni method adjusted P values.

449

450 **Figure 1** Genome-wide haplotype-block-based regional heritability mapping(HRHM)
451 results on MDD in GS:SFHS. A: Manhattan plot. Each point represents a haplotype
452 block. The location of the point is the mid-position of the haplotype block. B: qqplot
453 for the LRT. The LRT statistics distributed as a mixture of 0 and chi-squared (df=1)
454 distribution. C: zoom in region of the hit haplotype block region in Chromosome 20.
455 D: LD structure within the hit haplotype block in GS:SFHS. The block is located in
456 gene *TOX2*, it contains nine genotyped common SNPs(blue box) and five of them are
457 in high LD(red arrow) in GS:SFHS.

458

459 **Figure 2** Functional prediction of the hit haplotype block. A: functional annotation of
460 the hit block. The hit haplotype block (red bar on the left top showing the block and
461 blue bars showing the genotype SNPs in GS:SFHS) is located in the intron region and
462 a proportion of an adjacent exon of gene *TOX2*, overlapped with Fantom5 enhancers
463 and Transcription start sites, and regulatory-relevant histone modification
464 peaks(H3K27Ac and H3K4Me1). Within the block, 38 imputed SNPs were associated
465 with MDD, using SNP rs79645278(pink) as an example. This SNP is located in the
466 peak of active enhancer in astrocyte(highlighted with blue line). B,C: boxplots
467 showing tissue specific effect from SNPs that are both associated with MDD in
468 GS:SFHS and gene expression, using SNP rs79645278 as an example. B: the minor
469 allele of rs79645278 up-regulates the expression of a LncRNA RP1-269M15.3 in the
470 tissue 'Nucleus accumbens basal ganglia'. C: the minor allele of rs79645278 up-
471 regulates the expression of gene *TOX2* in Frontal cortex. Abbreviations: cerebellar
472 cortex (CRBL), frontal cortex (FCTX), hippocampus (HIPP), medulla (specifically

473 inferior olivary nucleus, MEDU), occipital cortex (specifically primary visual cortex,
474 OCTX), putamen (PUTM), substantia nigra (SNIG), thalamus (THAL), temporal
475 cortex (TCTX) and intralobular white matter (WHMT).
476
477 **Figure 3** Forest plot showing meta-analysis for single-SNP-based association test on
478 GS:SFHS and all UK/Ireland replication samples(four PGC2-MDD cohorts and UK
479 biobank), using SNP rs6093898 as an example.
480
481

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Table 1

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SNP information					Discovery: GS:SFHS				Replication1:UK Biobank				Replication2:PGC2-MDD (UK_Ireland)				Replication3:DS		
rs id	Chr	Pos	A1	A2	OR	logOR	se(logOR)	P	OR	logOR	se(logOR)	P	OR	logOR	se(logOR)	P	Beta	se	P
rs6017218	20	42555737	G(C)	T(A)	0.833	-0.183	0.041	2.44E-04	0.947	-0.055	0.030	0.068	0.842	-0.172	0.068	0.011	-0.013	0.005	0.007
rs6031242	20	42556096	G(C)	A(T)	0.832	-0.184	0.043	4.36E-04	0.948	-0.054	0.032	0.090	0.859	-0.153	0.071	0.032	-0.012	0.005	0.018
rs6031245	20	42559531	T(A)	C(G)	0.783	-0.244	0.045	2.30E-05	0.958	-0.043	0.035	0.225	0.843	-0.171	0.076	0.024	-0.015	0.006	0.011
rs6093898	20	42566577	G(C)	A(T)	0.783	-0.245	0.045	2.03E-05	0.958	-0.043	0.035	0.222	0.848	-0.165	0.075	0.028	-0.016	0.006	0.006
rs4812767	20	42568829	T(A)	C(G)	0.785	-0.242	0.045	2.57E-05	0.961	-0.040	0.035	0.253	0.840	-0.174	0.075	0.021	-0.016	0.006	0.006

Table 1. Single-SNP-based association test results for five MDD-associated SNPs in discovery and replication samples.

Table 2

Click here to download Table: Table 2.docx

Haplotype	Frequency	Beta(linear)	se(Beta(linear))	OR	logOR	se(logOR)	P	Adjusted P
TAGCGACCT	0.120	0.026	0.005	1.232	0.209	0.058	2.47E-06	1.73E-05 *
GGGTGGTCC	0.094	-0.024	0.006	0.792	-0.233	0.046	5.77E-05	4.04E-04 *
TAGCAACCT	0.118	-0.010	0.005	0.911	-0.093	0.045	6.10E-02	4.27E-01
TAGCGACTC	0.311	0.006	0.004	1.052	0.051	0.035	1.24E-01	8.71E-01
GAGCAACCT	0.012	-0.012	0.016	0.897	-0.109	0.131	4.60E-01	1.00E+00
TAGCAACCC	0.015	-0.010	0.014	0.916	-0.088	0.120	5.05E-01	1.00E+00
TATCGACTC	0.304	-0.002	0.004	0.980	-0.020	0.033	5.59E-01	1.00E+00

Table 2 Haplotype-based association test results for common haplotypes derived from the nine genotyped common SNPs in GS:SFHS. Adjusted P: Bonferroni method adjusted P values.

Figure 1
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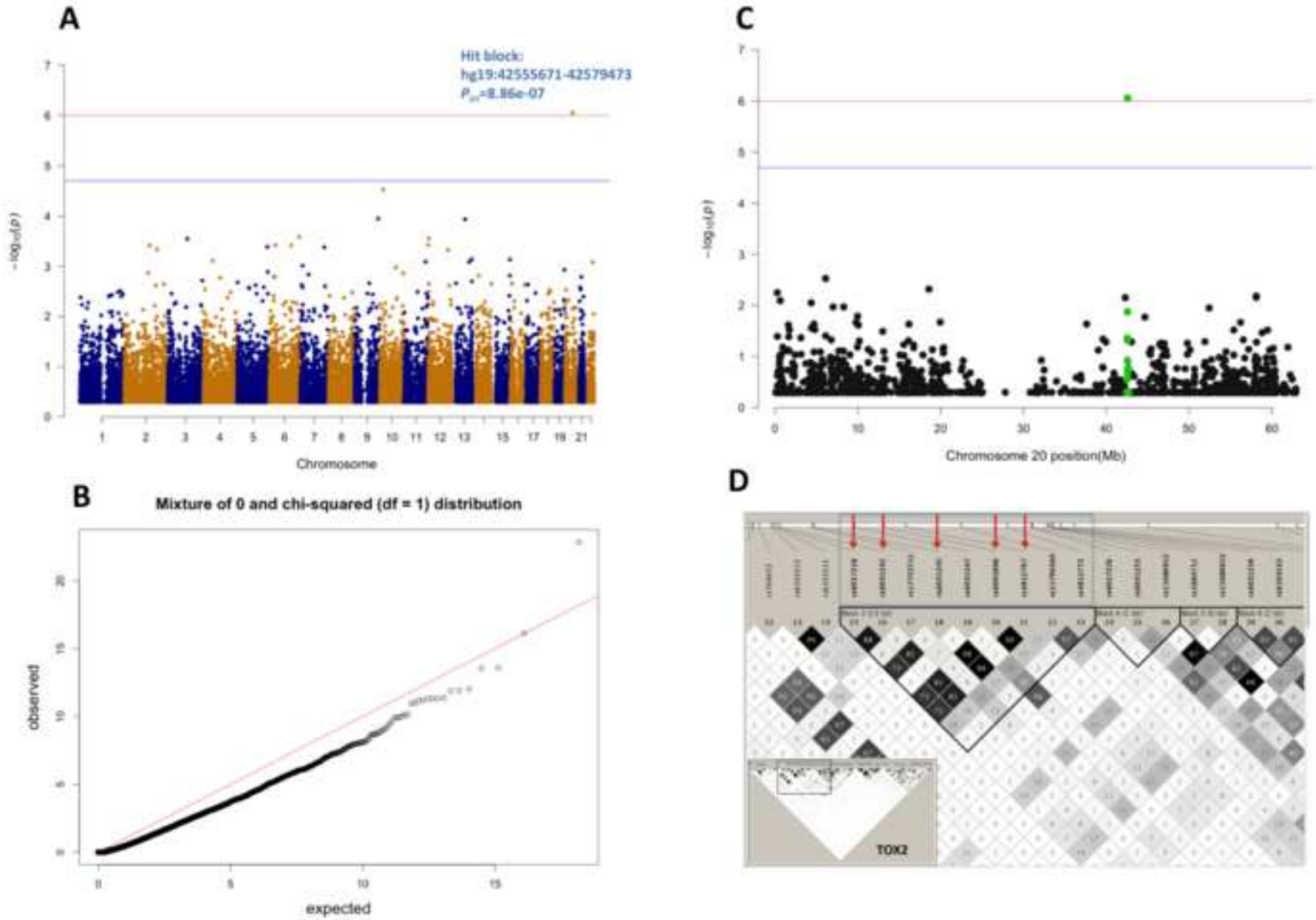

Figure 2

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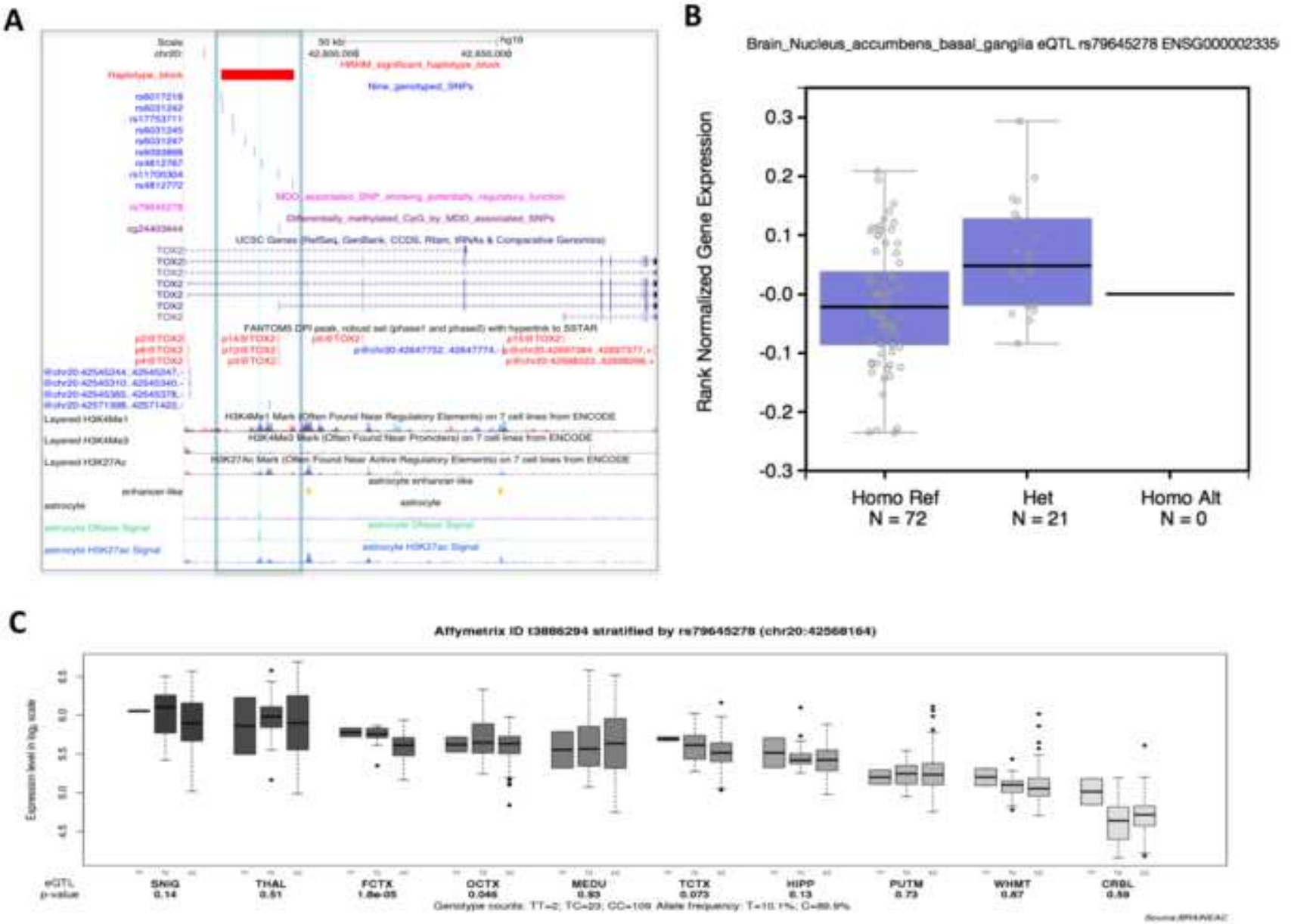

Figure 3
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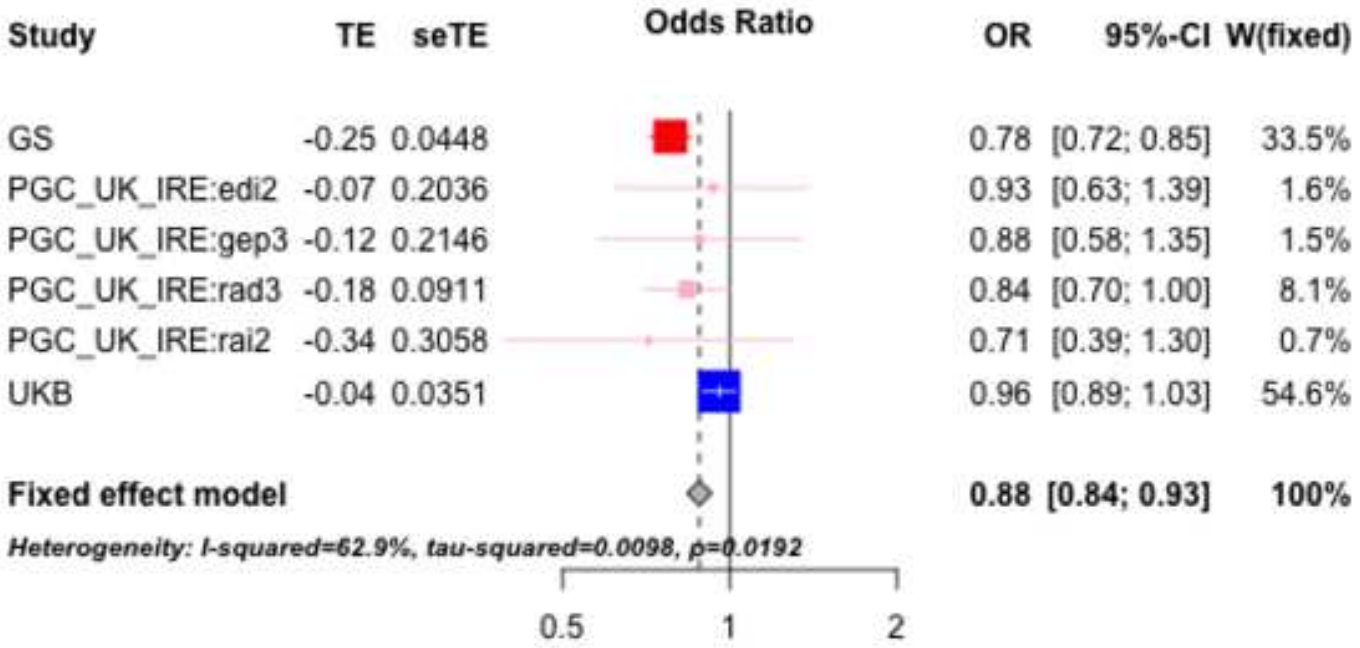

Supplementary information

Genome-wide regional heritability mapping identifies a locus within the TOX2 gene associated with Major Depressive Disorder

Text s1. Genotyping, quality control, imputation and phenotyping details in GS:SFHS dataset.

Genotyping data were generated using the Illumina Human OmniExpressExome -8- v1.0 array(1). Details of genotyping are described elsewhere(2). Quality control (QC) of genotyped SNPs used inclusion thresholds: missing SNPs per individual ≤2%, SNP genotype call rate ≥98%, minor allele frequency (MAF) >1% and Hardy-Weinberg equilibrium P value > 1×10^{-6}. In total, 561,125 genotyped autosomal SNPs passed QC criteria and were used in the subsequent analyses. Haplotypes were identified using SHAPEIT for phasing with the option --duohmm which refines the phasing by pedigree information(3). Imputation was performed using the Sanger Imputation server (https://imputation.sanger.ac.uk/) (HRC). After removing imputed SNPs with info score <0.8 and MAF < 0.01, 8,642,105 imputed SNPs remained.

Diagnosis of MDD: A structured clinical interview was used for the diagnosis of lifetime DSM-IV mood disorders (SCID)(4,5). Participants screened positive for mental health problems by endorsing one or more screening questions: "Have you ever seen anybody for emotional or psychiatric problems? or "Was there ever a time when you, or someone else, thought you should see someone because of the way you were feeling or acting" (prevalence=21.7%). At the end of the screening questions, participants screening positive were invited to continue to an interview using the SCID modules for mood disorders(4). Participants were excluded from the study when: (1) they screened positive for mental health problems but refused to undergo the structured clinical interview (N=507) or (2) those who fulfilled criteria for Bipolar Disorder (N=76). This left 19,896 genotyped participants with 2,659 MDD cases and 17,237 controls for the downstream analysis.

Text s2. Genotyping, quality control, imputation and phenotyping details in UK Biobank dataset.

UK Biobank recruited around 500,000 people aged between 40-69 years in 2006-2010 across the United Kingdom (UK Biobank 2011a)(6).

Genotyping and imputation: genotyping was performed for 152,729 UK Biobank participants using the Affymetrix UK Biobank Axiom array (N=102,750) and the Affymetrix UK BiLEVE Axiom array (N=49,979). Imputed data were generated by UK Biobank using a modified version of SHAPEIT for phasing and imputation was carried out using IMPUTE2, based on 1000 Genomes Phase 3 and UK10K haplotype panels(3,7). Additional QC was performed by removing SNPs with MAF < 0.01 and info score < 0.9. As the regional heritability analysis is highly computationally demanding, only those QC'ed imputed SNPs that could be mapped to the hapmap3 reference panel were used in downstream analyses(8). Additional filtering was performed for the genotyped subjects: 1) Non-white-British-participants were removed to reduce possible population stratification, this left 120,091 participants. 2) Subjects who were in both GS:SFHS and UK Biobank datasets were removed. 3) One of each pair of close relatives (relatedness > 0.05) of GS:SFSHS participants or the remained UK Biobank participants were removed from the UK Biobank sample. This left 116,981 genotyped participants.

MDD phenotype: The probable MDD phenotype was created based on the putative MDD definition established in Smith et al (2013) using responses to a touchscreen questionnaire (UK Biobank 2011b)(9), from self-report information, and from inpatient records via linkage to hospital episode data. In detail, All controls were assessed to be absent of depressive symptoms(9) by satisfying the following criteria: (1) The answers are 'No' for the following touch screen questionnaire at recruitment: "Looking back over your life, have you ever had a time when you were feeling depressed or down for at least a whole week?", "Have you ever seen a psychiatrist for nerves, anxiety, tension or depression?", "Have you ever had a time when you were uninterested in things or unable to enjoy the things you used to for at least a whole week?". (2) No primary diagnosis of ICD-10 Codes for mood disorders based on Hospital Episodes Data from UK bodies (English HES Data, Scottish Morbidity Register, Patient Episode Data). Cases satisfied the following criteria: (1) the answers of the following touchscreen questionnaire at recruitment are: 'Yes' for "Have you ever seen a psychiatrist for nerves, anxiety, tension or depression?"; either 'Yes' for "Looking back over your life, have you ever had a time when you were feeling depressed or down for at least a whole week?" and

‘More than two weeks’ for "How many weeks was the longest period when you were feeling depressed or down?" or ‘Yes’ for "Have you ever had a time when you were uninterested in things or unable to enjoy the things you used to for at least a whole week?" and ‘More than two weeks’ for "How many weeks was the longest period when you were uninterested in things or unable to enjoy the things you used to?". (2) Based on Hospital Episodes Data from UK bodies (English HES Data, Scottish Morbidity Register, Patient Episode Data, have a diagnosis of ICD-10 Codes for mood disorders. Additional sample filtering procedures were applied on the basis of self-report information and linked health records. Participants satisfying following criteria were excluded in the sample: (1)had been diagnosed to be bipolar disorder, multiple personality disorder, schizophrenia, autism, intellectual disability, Parkinson’s disease; or (2) self-reported bipolar disorder, schizophrenia, or Parkinson’s disease; or (3) bipolar disorder indicated by a touchscreen questionnaire assessment(9); or (4) had a prescription for antipsychotic or mood stabilising medication. Participants were excluded as controls if they (1) had a diagnosis of an anxiety disorder, a mood disorder, or major depressive disorder; or (2)had ever been prescribed antidepressant or anxiolytic medication; (3) self-reported depression; or (4) if they did not provide sufficient data to respond to these questions, or if their responses were intermediate between the control and case definitions described above. In total, 1,198,327 SNPs for 24,015 subjects with putative MDD phenotype available (8,143 cases and 15,872 controls) remained in downstream analyses.

Text s3. Genotyping, quality control, imputation and phenotyping details in PGC2-MDD dataset.

The Psychiatric Genomics Consortium provided lightly quality controlled (Missing-rate < 2%) individual genotypes (best guess) of imputed SNPs for participants from 22 cohorts in PGC2-MDD (Table s1).Subjects who overlapped with GS:SFHS and UK Biobank dataset (108 subjects) were removed. The remained data included 32,554 subjects of European ancestry (13,261 cases and 19,293 controls). We carried out additional QC for the imputed SNPs using the following inclusion thresholds: info score≥ 0.8, and MAF≥0.01. For each cohort, imputed SNPs that passed QC and could be mapped to the hapmap3 reference panel were used in downstream analyses (Table s1). All cases met DSM-IV criteria for life MDD, the majority of them were ascertained clinically. Most control samples were screened and participants with lifetime MDD were removed (Table s1). Consistent with earlier work(10,11), we grouped the 22 cohorts into 7 groups based on the country of ancestor information for regional heritability analysis (Table s1).

Text s4. Genome-wide haplotype-block-based Regional Heritability Mapping (HRHM)

To perform HRHM, estimated genome-wide recombination rates expressed as cM/Mb were downloaded from HapMap(phase II)(ftp://ftp.ncbi.nlm.nih.gov/hapmap). For each chromosome, we started from the first base position and expanded the region until it reached a recombination hot-spot where the estimated recombination rate was greater than 10 cM/Mb. This hot-spot marked the end of the region and the start of the next. This process continued until the end of chromosome was reached. Each region was considered to be a haplotype block. The genotyped SNPs were mapped to 49,637 haplotype-blocks across the genome and the regional heritability was estimated and tested for each of the haplotype-blocks. To test the regional heritability, it is necessary to appropriately account for the polygenic component and the pedigree structure in the analysis model. A standard model incorporates two genomic relationship matrices (GRM); a regional genomic relationship matrix (rGRM) estimated from SNPs in the haplotype block and a complement genomic relationship matrix (cGRM) estimated from all SNPs that are not included in in the haplotype block. These GRMs were jointly fitted in LMM:

$$\boldsymbol{Y = Xb + g_R + g_C + e}$$

$$\mathbf{Var}(\boldsymbol{Y_{random\_effect}}) = \boldsymbol{A_R\sigma_R^2 + A_C\sigma_C^2 + I\sigma_e^2}$$

$$\boldsymbol{h_R^2 = \sigma_R^2/\sigma_{Y_{random\_effect}}^2}$$

Where $\boldsymbol{Y}$ is a vector of MDD binary phenotypes, $\boldsymbol{b}$ is a vector of covariates fitted as fixed effects (i.e., age, age^2, sex, 20 principal components derived from the GRM created using all of the genotyped SNPs (fGRM)). $\boldsymbol{g_R}$ and $\boldsymbol{g_C}$ are the random genetic effects from the regional SNPs (in this study, the SNPs that were mapped to the haplotype-block) and the complement set of SNPs, respectively. $\boldsymbol{A_R}$ and $\boldsymbol{A_C}$ are the GRMs created from the regional SNPs and the complement set of SNPs, respectively. The variance explained by the rGRM variance component (regional heritability $\boldsymbol{h_R^2}$) is estimated using restricted estimated maximum likelihood (REML). The estimate is transformed from the observed scale to the liability scale assuming MDD prevalence of 0.13(5). A Log likelihood ratio test (LRT) is applied to test the significance of random effect represented in rGRM by comparing a model with both cGRM and an rGRM fitted against a model including the cGRM but without an rGRM fitted.

The two-GRM model, while providing an unbiased estimate of regional heritability, was highly computationally demanding. To improve the calculation efficiency, a pre-adjustment strategy was applied in the genome-wide HRHM. In detail, we created genomic relationship matrix using all of the genotyped SNPs (fGRM)(12) and pre-adjusted the random effect represented in the fGRM for the MDD phenotype using the 'polygenic' function in GenABEL(13). Residuals (pgresidualY option) were extracted from the model and used as the phenotype in the HRHM(13). The HRHM was performed by REACTA(14) using the following formula:

$$\boldsymbol{Y_{gresidual} = Xb + g_R + e}$$

$$\mathbf{Var}(\boldsymbol{Y_{gresidual\_random\_effect}}) = \boldsymbol{A_R\sigma_R^2 + I\sigma_e^2}$$

$$\boldsymbol{h_R^2 = \sigma_R^2/\sigma_{Y\ gresidual\_random\_effect}^2}$$

Where $\boldsymbol{Y_{gresidual}}$ is a vector of the residuals with fGRM being pre-adjusted, other parameters are the same as in the two-GRM-model. Multiple-testing-correction was performed for LRT statistics using the Bonferrroni method and the genome-wide significance threshold for HRHM was determined by the number of tests conducted (N_{blocks}=49,637)(15). The genome-wide significance threshold for P values from LRT is 1.01×10^{-6}. For haplotype-blocks that exceeded the genome-wide significant threshold, we re-tested the block using the two-GRM model to provide an accurate estimation of regional heritability in the target block. All the analyses were performed in REACTA(14,16).

Text s5 Single-haplotype-based association test

Full dataset containing both relatives and non-relatives: association tests were performed using GCTA-MLMA(12). For each participant, individual haplotypes were coded as 0, 1 or 2. In the linear mixed model, the effect from each haplotype on MDD phenotype was tested one at a time as a fixed effect, other covariates included age, age^2, sex and 20PCs. As in the single-SNP-based association test, two GRMs were fitted simultaneously as random effects in the model. Bonferroni multiple-testing correction was performed for the P values of each haplotype.

Unrelated dataset (N_{case}=997, $N_{control}$=6367): the dataset was generated by removing one of each pair of individuals with estimated relatedness larger than 0.025 by the function '--grm-cutoff 0.025' in GCTA(12). Association tests on the unrelated dataset were performed using GCTA-MLMA. Since the unrelated dataset contained no pair of individuals with a relatedness larger then 0.025, only the cGRM was fitted in the model.

Case-parent trios ($N_{case\text{-}parents\ trios}$=315): family-triad based logistic Bayesian Lasso (famLBL) is a method designed for testing the effects of haplotypes on diseases using SNP data using the triad family where the child is affected by the disease and both the child and parents have genotype and disease diagnosis information available(17). This method shrinks the coefficients of unassociated haplotypes and weighted toward rare haplotypes while retaining sufficient power for detecting common haplotype, allowing for more precise estimation of associated haplotypes(17). The confidence interval (CI) of OR and the Bayes Factor(BF) were given in the results and the BF threshold of 2 was applied (type I error rate ≤ 5%)(17).

Text s6. Functional annotation tools and analyses for MDD-associated SNPs.

(1)Regulomedb is a database providing annotations of known or predicted regulatory functions of non-coding variants. A score was provided for each SNP based on evidence from high-throughput experimental data as well as computational predictions and manual annotations to represent whether the SNP is likely to alter the binding of transcription factors(18).

(2) Genome Wide Annotation of VAriants (GWAVA) is a tool providing prediction of the functional influence of non-coding variants based on annotations of non-coding elements along with genome-wide properties, such as evolutionary conservation and GC-content(19).

(3) Genomic Evolutionary Rate Profiling (GERP) is a method providing estimates of evolutionary constraint with single variant resolution using maximum likelihood evolutionary rate estimation. A score threshold of 2 provides high sensitivity while still strongly enriching for truly constrained sites(20).

(4) Allelic effect on gene-expression (eQTL analysis).

In GTEX (http://www.gtexportal.org/home/datasets), tissue-specific SNP-*cis*-gene association test results were available from 11 brain tissues (a *cis* window was defined as +/- 1MB around the transcript start site (TSS).). The information available includes test statistics (a two tailed t-test) of the effect of the alternative allele relative to the reference allele on the expression of the gene for each SNP-gene pair in each tissue. We downloaded the 11 files and only extracted the records for the 38 SNPs. In BRAINEAC, tissue-specific SNP-*cis*-gene association test results were available from 10 brain tissues. P values from the tissue-specific *cis*-eQTL analyses were downloaded from http://caprica.genetics.kcl.ac.uk/BRAINEAC/. In results extracted from both GTEX and BRAINEAC, FDR multiple testing correction was applied to each tissue separately using the R package 'qvalue'(21)($N_{correction}=N_{cis\text{-}genes}*N_{snps}$).

(5) Allelic effect on DNA methylation in CpG loci (meQTL analysis).

Jaffe *et.al*.,(2016) identified meQTLs in frontal cortex using SNP-cis-CpG DNA methylation analysis on 258 adult control samples (age > 13)(22). Additionally, they also identified CpG loci that have differential DNA methylation level between fetal and postnatal life, which suggests a role of those loci in the early development stage in frontal cortex. We have extracted the records of the 38 SNPs from the downloaded summary statistics file of the SNP-cis-

CpG DNA methylation analysis, this included FDR values for each pair of associations and the estimate of the SNP effect (Beta). For the CpG loci that were significantly regulated by the 38 SNPs, we further checked the summary statistics file of the CpG loci differentially DNA methylated between fetal and postnatal life to see whether the regulated CpG is likely to play a role in the development of frontal cortex.

Group	Country of ascertainment	Cohort	N_snps	N_cases	N_controls	Study name	Case Definition	Screened Control
AUS	Australia	cof3	779887	120	126	COFAMS	MDD	yes
AUS	Australia	qi3c	832512	863	579	QIMR I317	MDD	yes
AUS	Australia	qi6c	1012990	499	590	QIMR I610	MDD	yes
AUS	Australia	qio2	781801	565	526	QIMR COEX	MDD	yes
GER	Germany	boma	1028670	586	1062	BOMA	MDD	no
GER	Germany	gsk2	1035814	879	860	GSK MPIP	rMDD	yes
GER	Germany	mmi2	828603	584	517	MPIP MARS 650	MDD	yes
GER	Germany	mmo4	1053857	264	371	MPIP MARS OMNIex	MDD	yes
GER	Germany	rage	1009799	322	227	RADIANT - German cases	rMDD	no
MIXED	Switzerland	col3	721251	506	1445	PsyCoLaus	MDD	yes
MIXED	Denmark	rde4	847865	133	516	RADIANT - Danish cases	rMDD	yes
NET	Netherlands	nes1	932158	1494	1602	NTR/NESDA	MDD	yes
NET	Netherlands	rot4	1029761	241	1028	Rotterdam	MDD	yes
SWE	Sweden	twg2	1089388	1097	2663	TwinGene	MDD	yes
UK_IRE	Scotland	edi2	950236	363	283	Edinburgh	rMDD	yes
UK_IRE	UK	gep3	1031973	472	2814	GENPOD/NewMeds	MDD	no
UK_IRE	UK	rad3	1040419	1835	1357	RADIANT	rMDD	yes
UK_IRE	Ireland	rai2	983451	109	339	RADIANT - Irish cases	rMDD	yes
USA	USA	grdg	1058400	471	470	DepGenesNetwork	MDD	yes
USA	USA	grnd	1063155	829	474	GenRED2	reoMDD	yes
USA	USA	i2b3	1034048	806	1066	Harvard i2b2	MDD	yes
USA	USA	rau2	1011577	223	378	RADIANT - US cases	rMDD	no

Table s1 Individual cohort and grouping information for 22 cohorts in PGC2-MDD. According to the country of ascertainment , the 22 cohorts were grouped into seven groups for the replication analysis.

rs ID	Chr	Pos	A1	A2	Freq	Beta(linear)	se(Beta(linear))	OR	logOR	se(logOR)	P	Adjusted P
rs6017218	20	42555737	G(C)	T(A)	0.126	-0.019	0.005	0.833	-0.183	0.041	2.44E-04	2.19E-03 *
rs6031242	20	42556096	G(C)	A(T)	0.113	-0.019	0.006	0.832	-0.184	0.043	4.36E-04	3.93E-03 *
rs17753711	20	42559319	A(T)	G(C)	0.305	-0.002	0.004	0.981	-0.019	0.033	5.76E-01	1.00E+00
rs6031245	20	42559531	T(A)	C(G)	0.096	-0.025	0.006	0.783	-0.244	0.045	2.30E-05	2.07E-04 *
rs6031247	20	42563647	A(T)	G(C)	0.147	-0.010	0.005	0.915	-0.089	0.041	4.89E-02	4.40E-01
rs6093898	20	42566577	G(C)	A(T)	0.097	-0.025	0.006	0.783	-0.245	0.045	2.03E-05	1.83E-04 *
rs4812767	20	42568829	T(A)	C(G)	0.096	-0.025	0.006	0.785	-0.242	0.045	2.57E-05	2.31E-04 *
rs11700304	20	42574362	C(G)	T(A)	0.374	-0.004	0.004	0.968	-0.032	0.031	3.17E-01	1.00E+00
rs4812772	20	42579051	T(A)	C(G)	0.261	0.008	0.004	1.072	0.070	0.037	4.19E-02	3.77E-01

Table s2 Single-SNP-based association test results for nine genotyped SNPs in the hit haplotype block in GS:SFHS. Beta(linear): estimates on the linear scale, OR was obtained using taylor series approximation. Adjusted P: Bonferroni method adjusted P value. *: significant results.

Haplotype	Freq	Beta(linear)	se(Beta(linear))	OR	logOR	se(logOR)	P	Adjusted P
TAGCGACCT	0.119	0.030	0.009	1.275	0.243	0.101	4.82E-04	3.85E-03 *
GGGCGACCT	0.010	0.047	0.028	1.440	0.364	0.371	9.26E-02	7.41E-01
GGGTGGTCC	0.094	-0.011	0.010	0.902	-0.103	0.079	2.47E-01	1.00E+00
TAGCGACTC	0.310	-0.005	0.006	0.957	-0.043	0.052	4.25E-01	1.00E+00
GAGCAACCT	0.012	-0.020	0.025	0.827	-0.190	0.196	4.26E-01	1.00E+00
TAGCAACCT	0.118	-0.007	0.009	0.942	-0.059	0.073	4.44E-01	1.00E+00
TAGCAACCC	0.015	-0.013	0.023	0.888	-0.119	0.188	5.79E-01	1.00E+00
TATCGACTC	0.307	-0.001	0.006	0.993	-0.007	0.053	9.00E-01	1.00E+00

Table s3 Haplotype-based association test results for common haplotypes derived from nine common SNPs in hit block on unrelated individual dataset of GS:SFHS. *: significant result.

Haplotype	Frequency	OR	Lower OR	Upper OR	BF
GGGTGGTCC	0.119	0.573	0.376	0.858	8.113 *
GAGCAACCT	0.014	0.577	0.191	1.348	0.806
GGGCGACTC	0.013	0.733	0.268	1.648	0.473
TAGCAACCC	0.018	1.083	0.559	2.185	0.282
TAGCAACCT	0.109	0.892	0.617	1.245	0.170
TAGCGACCT	0.120	1.126	0.824	1.569	0.167
TATCGACTC	0.285	0.888	0.687	1.151	0.155

Table s4 Haplotype-based association test results for common haplotypes derived from nine common SNPs in hit block on case-parents-trio of GS:SFHS. The BF threshold of 2 was applied for significance(type I error rate ≤5%). *: significant result.

rs id	Chr	Pos	A1	A2	Freq	Beta (linear)	Se (Beta(linear)	OR	logOR	se(logOR)	P	Adjusted P
rs4812766	20	42559149	A	G	0.096	-0.026	0.006	0.780	-0.248	0.045	1.72E-05	9.10E-04 *
rs11905261	20	42566120	C	A	0.096	-0.025	0.006	0.782	-0.246	0.045	1.99E-05	1.05E-03 *
rs78295570	20	42567815	T	C	0.097	-0.025	0.006	0.783	-0.245	0.045	2.00E-05	1.06E-03 *
rs6093898	20	42566577	G	A	0.097	-0.025	0.006	0.783	-0.245	0.045	2.03E-05	1.08E-03 *
rs6103524	20	42568152	G	A	0.097	-0.025	0.006	0.783	-0.245	0.045	2.03E-05	1.08E-03 *
rs77591323	20	42567817	C	T	0.097	-0.025	0.006	0.783	-0.245	0.045	2.10E-05	1.11E-03 *
rs6031245	20	42559531	T	C	0.096	-0.025	0.006	0.783	-0.244	0.045	2.30E-05	1.22E-03 *
rs75074505	20	42567941	G	C	0.097	-0.025	0.006	0.784	-0.243	0.045	2.31E-05	1.22E-03 *
rs1888982	20	42566009	A	G	0.097	-0.025	0.006	0.784	-0.243	0.045	2.31E-05	1.23E-03 *
rs916474	20	42563571	A	G	0.096	-0.025	0.006	0.784	-0.244	0.045	2.36E-05	1.25E-03 *
rs79225010	20	42557504	A	G	0.096	-0.025	0.006	0.784	-0.244	0.045	2.38E-05	1.26E-03 *
rs868981	20	42562557	A	G	0.096	-0.025	0.006	0.784	-0.243	0.045	2.46E-05	1.30E-03 *
rs11086910	20	42579473	A	G	0.095	-0.025	0.006	0.783	-0.244	0.045	2.49E-05	1.32E-03 *
rs4812767	20	42568829	T	C	0.096	-0.025	0.006	0.785	-0.242	0.045	2.57E-05	1.36E-03 *
rs944703	20	42564176	C	A	0.096	-0.025	0.006	0.785	-0.242	0.045	2.62E-05	1.39E-03 *
rs79645278	20	42568164	T	C	0.096	-0.025	0.006	0.785	-0.242	0.045	2.67E-05	1.41E-03 *
rs2002343	20	42570003	A	G	0.096	-0.025	0.006	0.785	-0.242	0.045	2.67E-05	1.41E-03 *
rs4383390	20	42572303	C	T	0.096	-0.025	0.006	0.785	-0.242	0.045	2.67E-05	1.41E-03 *
rs56852079	20	42562168	A	G	0.096	-0.025	0.006	0.786	-0.241	0.045	2.85E-05	1.51E-03 *
rs57150899	20	42562169	T	C	0.096	-0.025	0.006	0.786	-0.241	0.045	2.86E-05	1.52E-03 *
rs4812770	20	42575630	G	C	0.097	-0.025	0.006	0.789	-0.237	0.045	3.62E-05	1.92E-03 *
rs11907990	20	42556041	T	C	0.096	-0.024	0.006	0.790	-0.235	0.045	4.24E-05	2.24E-03 *
rs11906108	20	42556057	T	A	0.096	-0.024	0.006	0.790	-0.235	0.045	4.24E-05	2.24E-03 *
rs76623859	20	42555756	A	C	0.096	-0.024	0.006	0.791	-0.235	0.045	4.47E-05	2.37E-03 *
rs910909	20	42575799	G	A	0.096	-0.024	0.006	0.792	-0.233	0.045	4.84E-05	2.57E-03 *
rs910907	20	42576998	T	G	0.095	-0.024	0.006	0.793	-0.231	0.045	5.85E-05	3.10E-03 *
rs4812771	20	42575733	T	C	0.095	-0.024	0.006	0.793	-0.231	0.045	5.87E-05	3.11E-03 *
rs910908	20	42575841	A	G	0.095	-0.024	0.006	0.793	-0.231	0.045	5.87E-05	3.11E-03 *
rs6093895	20	42557501	A	G	0.118	-0.021	0.005	0.819	-0.200	0.042	1.08E-04	5.71E-03 *
rs6130482	20	42557999	C	T	0.118	-0.021	0.005	0.819	-0.200	0.042	1.09E-04	5.77E-03 *
rs743152	20	42569911	T	G	0.106	-0.022	0.006	0.811	-0.209	0.044	1.20E-04	6.34E-03 *
rs6017223	20	42571020	G	C	0.106	-0.022	0.006	0.814	-0.206	0.044	1.60E-04	8.47E-03 *
rs6017220	20	42564336	C	T	0.107	-0.021	0.006	0.816	-0.204	0.044	1.72E-04	9.12E-03 *
rs6103521	20	42566398	G	T	0.106	-0.021	0.006	0.816	-0.203	0.044	1.75E-04	9.28E-03 *
rs6103517	20	42558608	A	G	0.118	-0.020	0.005	0.825	-0.192	0.042	1.92E-04	1.02E-02 *
rs6017218	20	42555737	G	T	0.126	-0.019	0.005	0.833	-0.183	0.041	2.44E-04	1.29E-02 *
rs6031242	20	42556096	G	A	0.113	-0.019	0.006	0.832	-0.184	0.043	4.36E-04	2.31E-02 *
rs6103516	20	42556151	G	A	0.113	-0.019	0.006	0.834	-0.181	0.043	5.39E-04	2.86E-02 *
rs138949844	20	42570224	T	C	0.026	0.027	0.011	1.245	0.219	0.120	1.51E-02	1.00E+00
rs4812773	20	42579148	T	C	0.261	0.008	0.004	1.074	0.071	0.037	3.79E-02	1.00E+00
rs4812772	20	42579051	T	C	0.261	0.008	0.004	1.072	0.070	0.037	4.19E-02	1.00E+00
rs6031247	20	42563647	A	G	0.147	-0.010	0.005	0.915	-0.089	0.041	4.89E-02	1.00E+00
rs141048930	20	42567378	G	T	0.015	-0.027	0.014	0.767	-0.266	0.106	5.59E-02	1.00E+00
rs4812769	20	42574442	T	C	0.251	0.007	0.004	1.065	0.063	0.038	6.94E-02	1.00E+00

rs73118242	20	42568478	C	T	0.012	-0.017	0.016	0.853	-0.159	0.128	2.94E-01	1.00E+00
rs11700304	20	42574362	C	T	0.374	-0.004	0.004	0.968	-0.032	0.031	3.17E-01	1.00E+00
rs4812774	20	42579188	C	T	0.373	-0.004	0.004	0.969	-0.031	0.031	3.33E-01	1.00E+00
rs76571595	20	42572032	G	T	0.012	-0.014	0.016	0.879	-0.129	0.129	3.82E-01	1.00E+00
rs6031252	20	42573822	A	C	0.010	0.015	0.018	1.137	0.128	0.174	3.89E-01	1.00E+00
rs761919	20	42577362	C	T	0.372	-0.003	0.004	0.973	-0.028	0.031	3.93E-01	1.00E+00
rs73118230	20	42556503	C	T	0.012	-0.011	0.016	0.908	-0.097	0.131	5.06E-01	1.00E+00
rs79663350	20	42573034	A	G	0.028	0.001	0.011	1.009	0.009	0.095	9.20E-01	1.00E+00
rs8117150	20	42569690	A	G	0.015	0.001	0.015	1.007	0.007	0.130	9.59E-01	1.00E+00

Table s5 Results of Single-SNP-based association test on 53 imputed common SNPs in the hit block in GS:SFHS. Adjusted P: Bonferroni method adjusted P value. *: significant results.

rs id	Pos	Chr	A1	RegulomeDB	GWAVA-TSS	GERP
rs6017218	42555737	20	G	4	0.180	3.340
rs76623859	42555756	20	A	2b	0.180	-0.046
rs11907990	42556041	20	T	4	0.290	-2.900
rs11906108	42556057	20	T	4	0.240	-3.340
rs6031242	42556096	20	G	5	0.350	-5.020
rs6103516	42556151	20	G	5	0.120	-0.218
rs6093895	42557501	20	A	5	0.150	-1.560
rs79225010	42557504	20	A	5	0.130	0.780
rs6130482	42557999	20	C	5	0.090	-3.660
rs6103517	42558608	20	A	7	0.090	-0.633
rs4812766	42559149	20	A	5	0.180	1.860
rs6031245	42559531	20	T	7	0.360	-3.070
rs56852079	42562168	20	A	5	0.330	-4.140
rs57150899	42562169	20	T	5	0.190	-1.260
rs868981	42562557	20	A	5	0.330	2.250
rs916474	42563571	20	A	5	0.310	0.489
rs944703	42564176	20	C	5	0.180	-4.940
rs6017220	42564336	20	C	6	0.160	0.235
rs1888982	42566009	20	A	5	0.280	-2.420
rs11905261	42566120	20	C	5	0.300	0.964
rs6103521	42566398	20	G	4	0.240	-3.570
rs6093898	42566577	20	G	4	0.200	0.442
rs78295570	42567815	20	T	4	0.470	-5.270
rs77591323	42567817	20	C	4	0.530	-5.770
rs75074505	42567941	20	G	4	0.400	-1.570
rs6103524	42568152	20	G	4	0.510	2.290
rs79645278	**42568164**	**20**	**T**	**2b**	**0.500**	**2.310**
rs4812767	42568829	20	T	4	0.350	-0.751
rs743152	42569911	20	T	6	0.380	-1.980
rs2002343	42570003	20	A	7	0.330	-0.470
rs6017223	42571020	20	G	3a	0.510	-4.170
rs4383390	42572303	20	C	6	0.520	-2.190
rs4812770	42575630	20	G	5	0.120	1.370
rs4812771	42575733	20	T	7	0.180	2.090
rs910909	42575799	20	G	7	0.130	1.310
rs910908	42575841	20	A	6	0.120	-2.430
rs910907	42576998	20	T	5	0.200	-1.160
rs11086910	42579473	20	A	4	0.300	-1.820

Table s6 The functional prediction of 38 significant SNPs using regulomeDB score, GWAVA-Tss score and GERP score.

rs id	Pos	Chr	A1	Genename	Gene_pos	eQTL_beta	eQTL_P	eQTL_Qvalue
rs78295570	42567815	20	T	RP1-269M15.3	20_41818862-41830011	0.373132	2.35E-04	3.17E-02
rs77591323	42567817	20	C	RP1-269M15.3	20_41818862-41830011	0.373132	2.35E-04	3.17E-02
rs75074505	42567941	20	G	RP1-269M15.3	20_41818862-41830011	unsigned_0.373132	2.35E-04	3.17E-02
rs6103524	42568152	20	G	RP1-269M15.3	20_41818862-41830011	0.373132	2.35E-04	3.17E-02
rs1888982	42566009	20	A	RP1-269M15.3	20_41818862-41830011	0.373229	2.36E-04	3.17E-02
rs56852079	42562168	20	A	RP1-269M15.3	20_41818862-41830011	0.357319	2.52E-04	3.17E-02
rs910909	42575799	20	G	RP1-269M15.3	20_41818862-41830011	0.352567	2.94E-04	3.17E-02
rs11086910	42579473	20	A	RP1-269M15.3	20_41818862-41830011	0.381632	4.45E-04	4.10E-02
rs6093898	42566577	20	G	RP1-269M15.3	20_41818862-41830011	0.357227	4.89E-04	4.10E-02
rs743152	42569911	20	T	RP1-269M15.3	20_41818862-41830011	0.3497	8.18E-04	4.25E-02
rs6103521	42566398	20	G	RP1-269M15.3	20_41818862-41830011	0.336035	8.66E-04	4.25E-02
rs6017220	42564336	20	C	RP1-269M15.3	20_41818862-41830011	0.335674	8.85E-04	4.25E-02
rs11905261	42566120	20	C	RP1-269M15.3	20_41818862-41830011	0.338646	9.06E-04	4.25E-02
rs11906108	42556057	20	T	RP1-269M15.3	20_41818862-41830011	unsigned_0.35263	1.09E-03	4.25E-02
rs868981	42562557	20	A	RP1-269M15.3	20_41818862-41830011	0.31452	1.18E-03	4.25E-02
rs57150899	42562169	20	T	RP1-269M15.3	20_41818862-41830011	0.361794	1.41E-03	4.25E-02
rs11907990	42556041	20	T	RP1-269M15.3	20_41818862-41830011	0.347766	1.46E-03	4.25E-02
rs4383390	42572303	20	C	RP1-269M15.3	20_41818862-41830011	0.345133	1.59E-03	4.25E-02
rs6031245	42559531	20	T	RP1-269M15.3	20_41818862-41830011	0.345064	1.59E-03	4.25E-02
rs79645278	42568164	20	T	RP1-269M15.3	20_41818862-41830011	0.345064	1.59E-03	4.25E-02
rs4812767	42568829	20	T	RP1-269M15.3	20_41818862-41830011	0.345064	1.59E-03	4.25E-02
rs2002343	42570003	20	A	RP1-269M15.3	20_41818862-41830011	0.345064	1.59E-03	4.25E-02
rs4812771	42575733	20	T	RP1-269M15.3	20_41818862-41830011	0.345064	1.59E-03	4.25E-02
rs910908	42575841	20	A	RP1-269M15.3	20_41818862-41830011	0.345064	1.59E-03	4.25E-02
rs910907	42576998	20	T	RP1-269M15.3	20_41818862-41830011	0.345064	1.59E-03	4.25E-02
rs4812766	42559149	20	A	RP1-269M15.3	20_41818862-41830011	0.344928	1.60E-03	4.25E-02
rs79225010	42557504	20	A	RP1-269M15.3	20_41818862-41830011	0.344801	1.60E-03	4.25E-02
rs944703	42564176	20	C	RP1-269M15.3	20_41818862-41830011	0.344844	1.62E-03	4.25E-02
rs916474	42563571	20	A	RP1-269M15.3	20_41818862-41830011	0.344701	1.63E-03	4.25E-02
rs76623859	42555756	20	A	RP1-269M15.3	20_41818862-41830011	0.344566	1.71E-03	4.29E-02

Table s7 Significant results (Q value ≤ 0.05) from SNP-cis-gene-eQTL analysis using GTEX. The analysis were performed on 11 brain tissues in GTEX and significant results were obtained from the tissue 'Nucleus accumbens basal ganglia'. A1 is the tested allele, the same allele tested in single-SNP-based association test. Q value: Adjusted P values(FDR)

Gene Symbol	rs id	Chr	Pos	exprID	Gene_start	Gene_end	P	Q
TOX2,LOC100128170	rs4383390	20	42572303	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs2002343	20	42570003	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs868981	20	42562557	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs916474	20	42563571	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs944703	20	42564176	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs79225010	20	42557504	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs6031245	20	42559531	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs6093898	20	42566577	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs78295570	20	42567815	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs77591323	20	42567817	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs75074505	20	42567941	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs6103524	20	42568152	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs79645278	20	42568164	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs4812767	20	42568829	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs4812766	20	42559149	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs1888982	20	42566009	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs11905261	20	42566120	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs910909	20	42575799	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs910908	20	42575841	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs4812771	20	42575733	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs76623859	20	42555756	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs11907990	20	42556041	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs11906108	20	42556057	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs910907	20	42576998	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs743152	20	42569911	t3886294	42543004	42710556	1.60E-05	3.06E-04
TOX2,LOC100128170	rs6017223	20	42571020	t3886294	42543004	42710556	1.60E-05	3.06E-04
TOX2,LOC100128170	rs6103521	20	42566398	t3886294	42543004	42710556	1.60E-05	3.06E-04
TOX2,LOC100128170	rs6017220	20	42564336	t3886294	42543004	42710556	1.60E-05	3.06E-04
TOX2,LOC100128170	rs11086910	20	42579473	t3886294	42543004	42710556	1.80E-05	3.06E-04
TOX2,LOC100128170	rs6031242	20	42556096	t3886294	42543004	42710556	5.00E-06	3.06E-04
TOX2,LOC100128170	rs6103516	20	42556151	t3886294	42543004	42710556	5.00E-06	3.06E-04
TOX2,LOC100128170	rs4812770	20	42575630	t3886294	42543004	42710556	2.00E-05	3.29E-04
TOX2,LOC100128170	rs6017218	20	42555737	t3886294	42543004	42710556	2.10E-05	3.35E-04
TOX2,LOC100128170	rs6130482	20	42557999	t3886294	42543004	42710556	6.70E-05	1.04E-03
TOX2,LOC100128170	rs6093895	20	42557501	t3886294	42543004	42710556	6.90E-05	1.04E-03
TOX2,LOC100128170	rs57150899	20	42562169	t3886294	42543004	42710556	0.00026	3.70E-03
TOX2,LOC100128170	rs56852079	20	42562168	t3886294	42543004	42710556	0.00026	3.70E-03
TOX2,LOC100128170	rs6103517	20	42558608	t3886294	42543004	42710556	0.00078	1.08E-02

Table s8 Significant results (Q value ≤ 0.05) from SNP-cis-gene-eQTL analysis on Frontal cortex using BRAINEAC. The analysis was performed on 10 brain tissues in BRAINEAC and significant results were obtained from two tissues: Frontal cortex and Cerebellar cortex. A1 is the tested allele, the same allele tested in single-SNP-based association test. P: p values from SNP-cis-gene-eQTL analysis. Q: Adjusted P values(FDR)

Gene Symbol	Pos	Chr	rs id	exprID	chr	Gene_start	Gene_start	P	Q
C20orf62	42572303	20	rs4383390	t3906923	chr20	43080624	43093984	8.70E-04	2.34E-02
C20orf62	42566009	20	rs1888982	t3906923	chr20	43080624	43093984	8.70E-04	2.34E-02
C20orf62	42566120	20	rs11905261	t3906923	chr20	43080624	43093984	8.70E-04	2.34E-02
C20orf62	42570003	20	rs2002343	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42563571	20	rs916474	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42564176	20	rs944703	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42559531	20	rs6031245	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42566577	20	rs6093898	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42567815	20	rs78295570	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42567817	20	rs77591323	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42567941	20	rs75074505	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42568152	20	rs6103524	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42568164	20	rs79645278	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42568829	20	rs4812767	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42562557	20	rs868981	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42559149	20	rs4812766	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42575630	20	rs4812770	t3906923	chr20	43080624	43093984	8.50E-04	2.34E-02
C20orf62	42575841	20	rs910908	t3906923	chr20	43080624	43093984	8.70E-04	2.34E-02
C20orf62	42575733	20	rs4812771	t3906923	chr20	43080624	43093984	8.70E-04	2.34E-02
C20orf62	42575799	20	rs910909	t3906923	chr20	43080624	43093984	8.70E-04	2.34E-02
C20orf62	42555756	20	rs76623859	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42556041	20	rs11907990	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42556057	20	rs11906108	t3906923	chr20	43080624	43093984	8.80E-04	2.34E-02
C20orf62	42564336	20	rs6017220	t3906923	chr20	43080624	43093984	8.90E-04	2.34E-02
C20orf62	42566398	20	rs6103521	t3906923	chr20	43080624	43093984	8.90E-04	2.34E-02
C20orf62	42569911	20	rs743152	t3906923	chr20	43080624	43093984	9.00E-04	2.34E-02
C20orf62	42571020	20	rs6017223	t3906923	chr20	43080624	43093984	9.00E-04	2.34E-02
C20orf62	42576998	20	rs910907	t3906923	chr20	43080624	43093984	8.70E-04	2.34E-02
C20orf62	42557504	20	rs79225010	t3906923	chr20	43080624	43093984	9.10E-04	2.34E-02
C20orf62	42579473	20	rs11086910	t3906923	chr20	43080624	43093984	8.70E-04	2.34E-02
C20orf62	42562169	20	rs57150899	t3906923	chr20	43080624	43093984	2.80E-04	2.34E-02
C20orf62	42562168	20	rs56852079	t3906923	chr20	43080624	43093984	2.80E-04	2.34E-02
C20orf62	42556096	20	rs6031242	t3906923	chr20	43080624	43093984	1.10E-03	2.66E-02
C20orf62	42556151	20	rs6103516	t3906923	chr20	43080624	43093984	1.10E-03	2.66E-02
C20orf62	42558608	20	rs6103517	t3906923	chr20	43080624	43093984	2.70E-03	6.35E-02

Table s9 Significant results (Q value ≤ 0.05) from SNP-cis-gene-eQTL analysis on Cerebellar cortex using BRAINEAC. The analysis was performed on 10 brain tissues in BRAINEAC and significant results were obtained from two tissues: Frontal cortex and Cerebellar cortex. A1 is the tested allele, the same allele tested in single-SNP-based association test. P: p values from SNP-cis-gene-eQTL analysis. Q: Adjusted P values(FDR).

snpChr	snpPos	snpRsNum	snpCounted	cpg	methChr	methPos	beta	pvalue	FDR
chr20	42563571	rs916474	A	cg24403644	chr20	42574624	-0.02	9.99E-08	2.80E-06
chr20	42564176	rs944703	C	cg24403644	chr20	42574624	-0.02	1.66E-07	4.48E-06
chr20	42566009	rs1888982	A	cg24403644	chr20	42574624	-0.01	2.58E-08	7.94E-07
chr20	42566120	rs11905261	C	cg24403644	chr20	42574624	-0.01	2.31E-07	6.09E-06
chr20	42566577	rs6093898	G	cg24403644	chr20	42574624	-0.01	9.27E-07	2.20E-05
chr20	42567815	rs78295570	T	cg24403644	chr20	42574624	-0.01	2.88E-07	7.47E-06
chr20	42567817	rs77591323	C	cg24403644	chr20	42574624	-0.01	2.88E-07	7.47E-06
chr20	42567941	rs75074505	G	cg24403644	chr20	42574624	-0.01	6.72E-07	1.63E-05
chr20	42568152	rs6103524	G	cg24403644	chr20	42574624	-0.01	2.88E-07	7.47E-06
chr20	42568164	rs79645278	T	cg24403644	chr20	42574624	-0.02	1.00E-07	2.81E-06
chr20	42568829	rs4812767	T	cg24403644	chr20	42574624	-0.02	1.00E-07	2.81E-06
chr20	42570003	rs2002343	A	cg24403644	chr20	42574624	-0.01	1.14E-06	2.65E-05
chr20	42572303	rs4383390	C	cg24403644	chr20	42574624	-0.01	1.14E-06	2.65E-05
chr20	42575630	rs4812770	G	cg24403644	chr20	42574624	-0.02	3.17E-07	8.17E-06
chr20	42575733	rs4812771	T	cg24403644	chr20	42574624	-0.02	2.68E-07	7.00E-06
chr20	42575799	rs910909	G	cg24403644	chr20	42574624	-0.01	6.87E-05	1.09E-03
chr20	42575841	rs910908	A	cg24403644	chr20	42574624	-0.02	3.41E-07	8.75E-06
chr20	42576998	rs910907	T	cg24403644	chr20	42574624	-0.02	9.77E-08	2.75E-06
chr20	42579473	rs11086910	A	cg24403644	chr20	42574624	-0.01	5.12E-08	1.50E-06

Table s10 Significant results of meQTL SNPs for CpG locus cg24403644 (FDR ≤ 0.05) from SNP-cis-CpG DNA methylation analysis on Frontal cortex.

Replication Sample	Group	P_{lrt}	h_R^2	se(h_R^2)	h_C^2	se(h_C^2)
PGC2-MDD	Combined 22	0.500	0.000	0.000	0.139	0.012
PGC2-MDD	AUS	the matrix V becomes negative-definite				
PGC2-MDD	GER	0.415	0.000	0.001	0.357	0.064
PGC2-MDD	MIXED	0.500	0.000	0.002	0.157	0.159
PGC2-MDD	NET	0.500	0.000	0.001	0.351	0.081
PGC2-MDD	SWE	0.068	0.003	0.004	0.341	0.115
PGC2-MDD	UK-Ireland	0.049*	0.001	0.001	0.227	0.051
PGC2-MDD	USA	0.500	0.000	0.001	0.252	0.079
UK Biobank	UK Biobank	0.151	0.000	0.000	0.130	0.019

Table s11 Regional heritability estimates of the hit block in seven groups and the combined (22 cohorts) in PGC2-MDD and UK Biobank. h_R^2: regional heritability. P_{lrt}: P value of LRT for h_R^2.

rs id	Pos	Chr	Cohort	A1	OR	logOR	se(logOR)	P
rs6017218	42555737	20	boma	G	1.006	0.006	0.120	0.960
rs6017218	42555737	20	cof3	G	0.746	-0.293	0.279	0.294
rs6017218	42555737	20	gep3	G	0.916	-0.087	0.180	0.626
rs6017218	42555737	20	grdg	G	0.966	-0.035	0.139	0.801
rs6017218	42555737	20	grnd	G	0.939	-0.063	0.131	0.632
rs6017218	42555737	20	gsk2	G	0.924	-0.079	0.103	0.446
rs6017218	42555737	20	i2b3	G	1.160	0.148	0.100	0.139
rs6017218	42555737	20	mmi2	G	1.150	0.140	0.130	0.284
rs6017218	42555737	20	mmo4	G	1.089	0.085	0.213	0.690
rs6017218	42555737	20	qi3c	G	0.968	-0.032	0.114	0.776
rs6017218	42555737	20	qi6c	G	1.019	0.019	0.131	0.886
rs6017218	42555737	20	qio2	G	1.168	0.155	0.136	0.252
rs6017218	42555737	20	rad3	G	0.824	-0.193	0.076	0.011 *
rs6017218	42555737	20	rage	G	1.020	0.020	0.208	0.924
rs6017218	42555737	20	rai2	G	0.903	-0.102	0.265	0.699
rs6017218	42555737	20	rau2	G	1.455	0.375	0.188	0.046 *
rs6017218	42555737	20	rde4	G	1.382	0.324	0.212	0.127
rs6017218	42555737	20	rot4	G	0.770	-0.262	0.164	0.111
rs6017218	42555737	20	twg2	G	1.092	0.088	0.076	0.248
rs6031242	42556096	20	boma	G	1.033	0.032	0.122	0.791
rs6031242	42556096	20	cof3	G	0.638	-0.449	0.296	0.129
rs6031242	42556096	20	gep3	G	0.963	-0.038	0.185	0.838
rs6031242	42556096	20	grdg	G	0.918	-0.086	0.148	0.564
rs6031242	42556096	20	grnd	G	0.938	-0.064	0.137	0.643
rs6031242	42556096	20	gsk2	G	0.921	-0.082	0.105	0.435
rs6031242	42556096	20	i2b3	G	1.041	0.040	0.107	0.708
rs6031242	42556096	20	mmi2	G	1.182	0.167	0.144	0.246
rs6031242	42556096	20	mmo4	G	1.239	0.214	0.221	0.333
rs6031242	42556096	20	qi3c	G	0.950	-0.052	0.123	0.674
rs6031242	42556096	20	qi6c	G	1.090	0.086	0.141	0.540
rs6031242	42556096	20	qio2	G	1.154	0.143	0.143	0.317
rs6031242	42556096	20	rad3	G	0.841	-0.174	0.080	0.031 *
rs6031242	42556096	20	rage	G	1.057	0.055	0.214	0.796
rs6031242	42556096	20	rai2	G	0.854	-0.158	0.278	0.569
rs6031242	42556096	20	rau2	G	1.344	0.296	0.198	0.135
rs6031242	42556096	20	rde4	G	1.287	0.252	0.216	0.244
rs6031242	42556096	20	rot4	G	0.652	-0.428	0.179	0.017 *
rs6031242	42556096	20	twg2	G	1.075	0.072	0.079	0.362
rs6031245	42559531	20	boma	T	0.999	-0.001	0.137	0.997
rs6031245	42559531	20	cof3	T	0.626	-0.468	0.347	0.177
rs6031245	42559531	20	col3	T	0.901	-0.104	0.140	0.458
rs6031245	42559531	20	edi2	T	0.895	-0.111	0.204	0.586
rs6031245	42559531	20	gep3	T	0.892	-0.115	0.215	0.592
rs6031245	42559531	20	grdg	T	0.964	-0.037	0.163	0.821

rs6031245	42559531	20	grnd	T	0.925	-0.078	0.154	0.615
rs6031245	42559531	20	gsk2	T	0.930	-0.073	0.122	0.549
rs6031245	42559531	20	i2b3	T	1.080	0.077	0.122	0.529
rs6031245	42559531	20	mmi2	T	0.978	-0.023	0.157	0.885
rs6031245	42559531	20	mmo4	T	1.259	0.230	0.249	0.355
rs6031245	42559531	20	nes1	T	1.050	0.049	0.092	0.597
rs6031245	42559531	20	qi3c	T	1.005	0.005	0.131	0.970
rs6031245	42559531	20	qi6c	T	1.161	0.149	0.155	0.336
rs6031245	42559531	20	qio2	T	1.086	0.083	0.166	0.619
rs6031245	42559531	20	rad3	T	0.837	-0.178	0.091	0.052
rs6031245	42559531	20	rage	T	1.355	0.304	0.263	0.248
rs6031245	42559531	20	rai2	T	0.715	-0.336	0.306	0.272
rs6031245	42559531	20	rau2	T	1.323	0.280	0.210	0.183
rs6031245	42559531	20	rde4	T	1.100	0.095	0.239	0.690
rs6031245	42559531	20	rot4	T	0.551	-0.596	0.225	0.008 *
rs6031245	42559531	20	twg2	T	1.133	0.125	0.089	0.162
rs6093898	42566577	20	boma	G	0.968	-0.032	0.137	0.814
rs6093898	42566577	20	cof3	G	0.672	-0.397	0.343	0.247
rs6093898	42566577	20	col3	G	0.898	-0.107	0.140	0.445
rs6093898	42566577	20	edi2	G	0.933	-0.069	0.204	0.735
rs6093898	42566577	20	gep3	G	0.884	-0.124	0.215	0.564
rs6093898	42566577	20	grdg	G	0.947	-0.055	0.163	0.737
rs6093898	42566577	20	grnd	G	0.944	-0.058	0.154	0.710
rs6093898	42566577	20	gsk2	G	0.923	-0.080	0.122	0.512
rs6093898	42566577	20	i2b3	G	1.094	0.090	0.121	0.460
rs6093898	42566577	20	mmi2	G	0.985	-0.015	0.157	0.922
rs6093898	42566577	20	mmo4	G	1.137	0.128	0.247	0.603
rs6093898	42566577	20	nes1	G	1.041	0.040	0.090	0.657
rs6093898	42566577	20	qi3c	G	1.027	0.027	0.135	0.844
rs6093898	42566577	20	qi6c	G	1.161	0.149	0.155	0.335
rs6093898	42566577	20	qio2	G	1.129	0.121	0.164	0.460
rs6093898	42566577	20	rad3	G	0.838	-0.176	0.091	0.053
rs6093898	42566577	20	rage	G	1.371	0.316	0.262	0.228
rs6093898	42566577	20	rai2	G	0.715	-0.336	0.306	0.272
rs6093898	42566577	20	rau2	G	1.265	0.235	0.208	0.258
rs6093898	42566577	20	rde4	G	1.069	0.067	0.234	0.776
rs6093898	42566577	20	rot4	G	0.579	-0.546	0.222	0.014 *
rs6093898	42566577	20	twg2	G	1.131	0.123	0.089	0.167
rs4812767	42568829	20	boma	T	0.973	-0.027	0.138	0.845
rs4812767	42568829	20	cof3	T	0.620	-0.478	0.347	0.168
rs4812767	42568829	20	col3	T	0.901	-0.104	0.140	0.459
rs4812767	42568829	20	edi2	T	0.924	-0.079	0.204	0.700
rs4812767	42568829	20	gep3	T	0.889	-0.117	0.214	0.584
rs4812767	42568829	20	grdg	T	0.954	-0.047	0.163	0.774
rs4812767	42568829	20	grnd	T	0.896	-0.110	0.155	0.480

rs4812767	42568829	20	gsk2	T	0.930	-0.073	0.122	0.549
rs4812767	42568829	20	i2b3	T	1.098	0.093	0.122	0.444
rs4812767	42568829	20	mmi2	T	0.968	-0.033	0.158	0.835
rs4812767	42568829	20	mmo4	T	1.336	0.290	0.251	0.249
rs4812767	42568829	20	nes1	T	1.047	0.046	0.092	0.619
rs4812767	42568829	20	qi3c	T	1.023	0.023	0.133	0.865
rs4812767	42568829	20	qi6c	T	1.161	0.149	0.155	0.335
rs4812767	42568829	20	qio2	T	1.128	0.120	0.165	0.467
rs4812767	42568829	20	rad3	T	0.827	-0.190	0.092	0.038 *
rs4812767	42568829	20	rage	T	1.371	0.316	0.262	0.228
rs4812767	42568829	20	rai2	T	0.728	-0.317	0.306	0.300
rs4812767	42568829	20	rau2	T	1.283	0.249	0.208	0.232
rs4812767	42568829	20	rde4	T	1.069	0.067	0.234	0.776
rs4812767	42568829	20	rot4	T	0.552	-0.595	0.225	0.008 *
rs4812767	42568829	20	twg2	T	1.144	0.135	0.089	0.133

Table s12 Single-SNP-based association tests for five SNPs in individual cohorts PGC2-MDD.

rs id	group	OR	logOR	se(logOR)	lower(OR)	Upper(OR)	p
rs4812767	PGC_AUS	1.057	0.056	0.084	0.897	1.246	0.507
rs4812767	PGC_GER	1.008	0.008	0.072	0.875	1.162	0.912
rs4812767	PGC_MIXED	0.943	-0.059	0.120	0.745	1.194	0.625
rs4812767	PGC_NET	0.955	-0.046	0.085	0.808	1.129	0.590
rs4812767	PGC_SWE	1.144	0.135	0.089	0.960	1.363	0.133
rs4812767	PGC_UK_IRE	0.840	-0.174	0.075	0.725	0.974	0.021*
rs4812767	PGC_USA	1.034	0.034	0.077	0.889	1.202	0.662
rs4812767	UKB+PGC_UK_IRE	0.938	-0.064	0.032	0.881	0.998	0.044*
rs4812767	PGC_combined22	1.002	0.002	0.029	0.946	1.061	0.956
rs6017218	PGC_AUS	1.016	0.016	0.070	0.886	1.166	0.817
rs6017218	PGC_GER	1.013	0.013	0.061	0.899	1.142	0.827
rs6017218	PGC_MIXED	1.382	0.324	0.212	0.912	2.095	0.127
rs6017218	PGC_NET	0.770	-0.262	0.164	0.558	1.062	0.111
rs6017218	PGC_SWE	1.092	0.088	0.076	0.941	1.268	0.248
rs6017218	PGC_UK_IRE	0.842	-0.172	0.068	0.737	0.961	0.011*
rs6017218	PGC_USA	1.087	0.084	0.065	0.958	1.234	0.197
rs6017218	UKB+PGC_UK_IRE	0.928	-0.074	0.028	0.880	0.980	0.007*
rs6017218	PGC_combined22	1.002	0.002	0.031	0.946	1.061	0.956
rs6031242	PGC_AUS	1.016	0.016	0.075	0.877	1.177	0.835
rs6031242	PGC_GER	1.034	0.034	0.064	0.913	1.171	0.596
rs6031242	PGC_MIXED	1.287	0.252	0.216	0.842	1.967	0.244
rs6031242	PGC_NET	0.652	-0.428	0.179	0.458	0.926	0.017*
rs6031242	PGC_SWE	1.075	0.072	0.079	0.920	1.256	0.362
rs6031242	PGC_UK_IRE	0.858	-0.153	0.071	0.747	0.987	0.032*
rs6031242	PGC_USA	1.018	0.018	0.069	0.890	1.165	0.794
rs6031242	UKB+PGC_UK_IRE	0.932	-0.070	0.029	0.881	0.987	0.016*
rs6031242	PGC_combined22	0.989	-0.011	0.031	0.931	1.051	0.720
rs6031245	PGC_AUS	1.040	0.039	0.083	0.883	1.224	0.642
rs6031245	PGC_GER	1.012	0.012	0.072	0.878	1.166	0.872
rs6031245	PGC_MIXED	0.949	-0.053	0.121	0.748	1.202	0.662
rs6031245	PGC_NET	0.957	-0.044	0.085	0.810	1.131	0.607
rs6031245	PGC_SWE	1.133	0.125	0.089	0.951	1.350	0.162
rs6031245	PGC_UK_IRE	0.843	-0.171	0.075	0.727	0.977	0.024*
rs6031245	PGC_USA	1.041	0.040	0.077	0.895	1.211	0.600
rs6031245	UKB+PGC_UK_IRE	0.937	-0.066	0.032	0.880	0.997	0.040*
rs6031245	PGC_combined22	0.991	-0.009	0.031	0.932	1.054	0.767
rs6093898	PGC_AUS	1.064	0.062	0.084	0.903	1.254	0.460
rs6093898	PGC_GER	0.995	-0.005	0.072	0.863	1.146	0.941
rs6093898	PGC_MIXED	0.941	-0.061	0.120	0.743	1.191	0.611
rs6093898	PGC_NET	0.957	-0.044	0.084	0.812	1.128	0.603
rs6093898	PGC_SWE	1.131	0.123	0.089	0.950	1.347	0.167
rs6093898	PGC_UK_IRE	0.848	-0.165	0.075	0.732	0.983	0.028*
rs6093898	PGC_USA	1.042	0.041	0.077	0.897	1.211	0.589

rs6093898	UKB+PGC_UK_IRE	0.937	-0.065	0.032	0.881	0.998	0.042*
rs6093898	PGC_combined22	0.991	-0.009	0.031	0.932	1.054	0.772

Table s13 Meta-analysis results for five SNPs in groups and combined sample of PGC and all UK replication samples.

Gene-symbol	Chromosome	r
RP1-269M15.3	20	1
PTPRT	20	0.833
LRFN5	14	0.78
GRM7	3	0.77
EPHA10	1	0.746
RP11-497E19.1	14	0.739
RP11-586D19.1	4	0.737
FAM78B	1	0.736
RP11-497E19.2	14	0.736
DLEU7	13	0.733
C9orf91	9	0.73
CACNA2D3	3	0.72
FBXL2	3	0.716
LRRC4C	11	0.716
FAM84A	2	0.711
RFPL1-AS1	22	0.711
DCBLD1	6	0.709
RFPL1	22	0.709
CHSY3	5	0.705
PPFIA2	12	0.704
ARMCX2	X	0.701
RP11-133K1.2	15	0.7

Table s14. Genes showing similar expression patterns with RP1-269M15.3 (r≥0.7) in development brain tissues in BRAINSPAN. r: correlation of gene expression with RP1-269M15.3. The data was downloaded from BrainSpan: Atlas of the Developing Human Brain [Internet]. Funded by ARRA Awards 1RC2MH089921-01, 1RC2MH090047-01, and 1RC2MH089929-01. © 2011. Available from: http://developinghumanbrain.org.

Gene-symbol	Chromosome	r
TOX2	20	1
GPR123	10	0.808
KIF17	1	0.761
CRH	8	0.754
SYT17	16	0.748
VWC2L	2	0.744
ARID5B	10	0.73
CPNE7	16	0.73
NECAB2	16	0.719
NTNG1	1	0.716
STBD1	4	0.713
CYB561	17	0.707
FAM127A	X	0.705
RP13-137A17.4	10	0.702

Table s15 Genes showing similar expression patterns with *TOX2* (r≥0.7) in development brain tissues in BRAINSPAN. r: correlation of gene expression with *TOX2*. The data was downloaded from BrainSpan: Atlas of the Developing Human Brain [Internet]. Funded by ARRA Awards 1RC2MH089921-01, 1RC2MH090047-01, and 1RC2MH089929-01. © 2011. Available from: http://developinghumanbrain.org

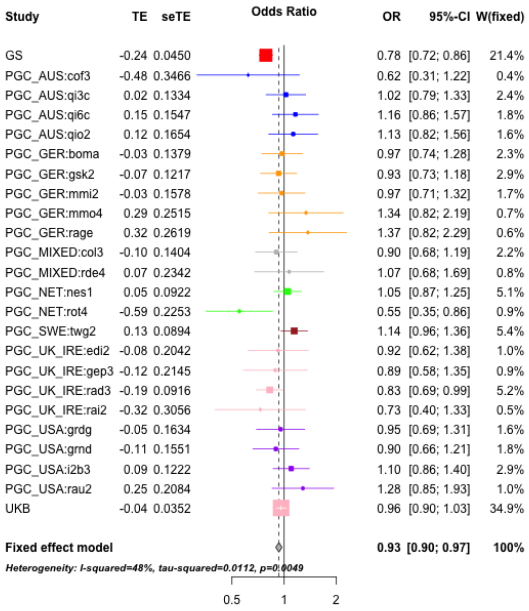

Figure s1A Forest plots of meta-analysis of GS:SFHS, PGC2-MDD individual cohorts and UK Biobank for rs4812767.

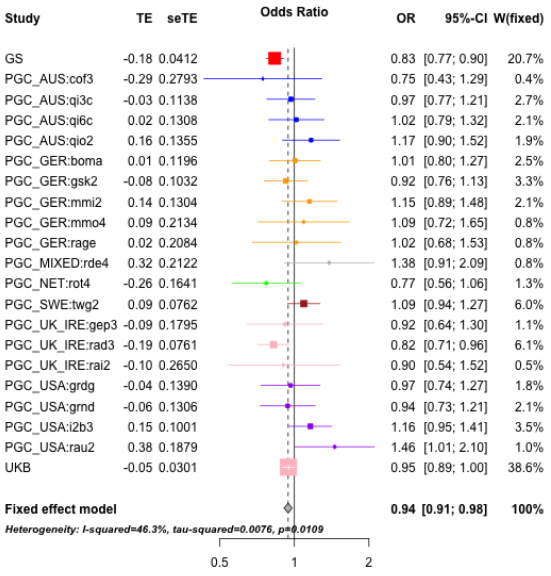

Figure s1B Forest plots of meta-analysis of GS:SFHS, PGC2-MDD individual cohorts and UK Biobank for rs6017218.

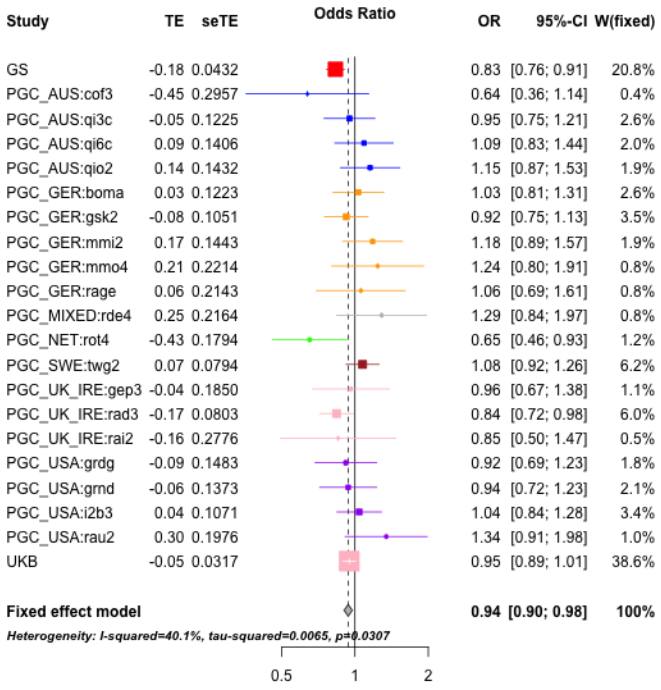

Figure s1C Forest plots of meta-analysis of GS:SFHS, PGC2-MDD individual cohorts and UK Biobank for rs6031242.

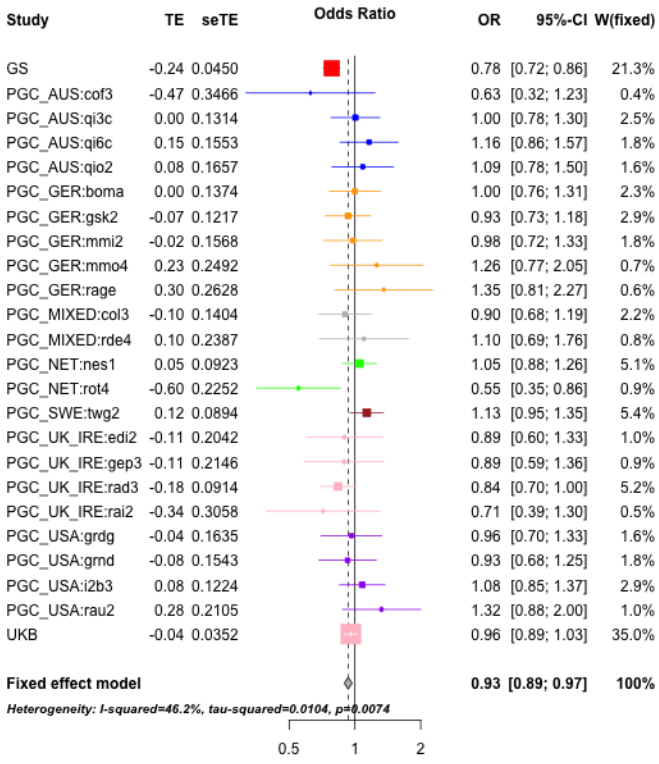

Figure s1D Forest plots of meta-analysis of GS:SFHS, PGC2-MDD individual cohorts and UK Biobank for rs6031245.

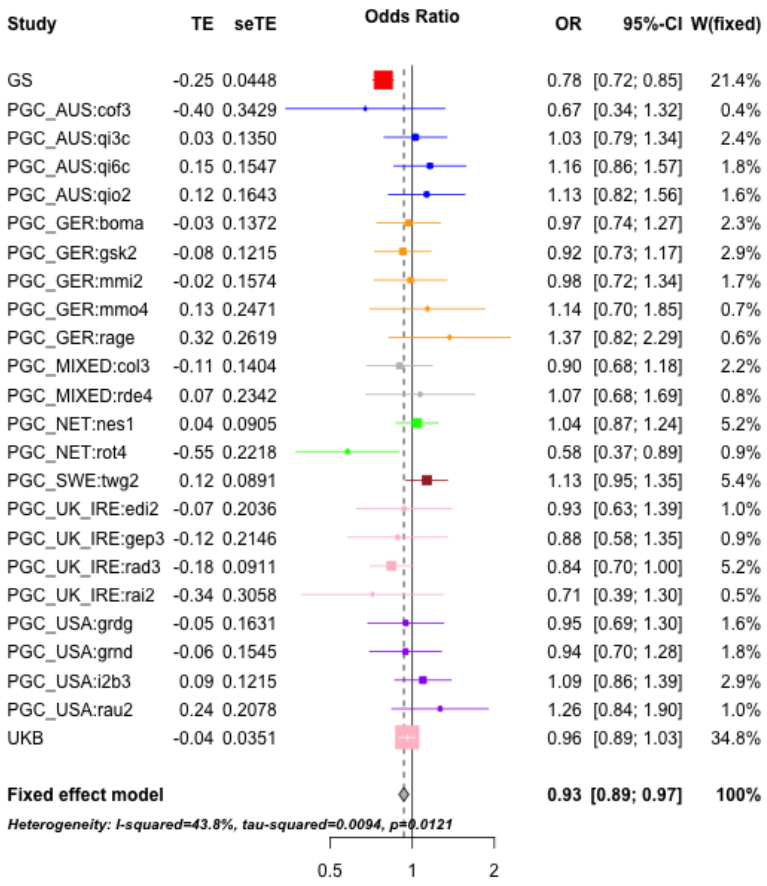

Figure s1E Forest plots of meta-analysis of GS:SFHS, PGC2-MDD individual cohorts and UK Biobank for rs6093898

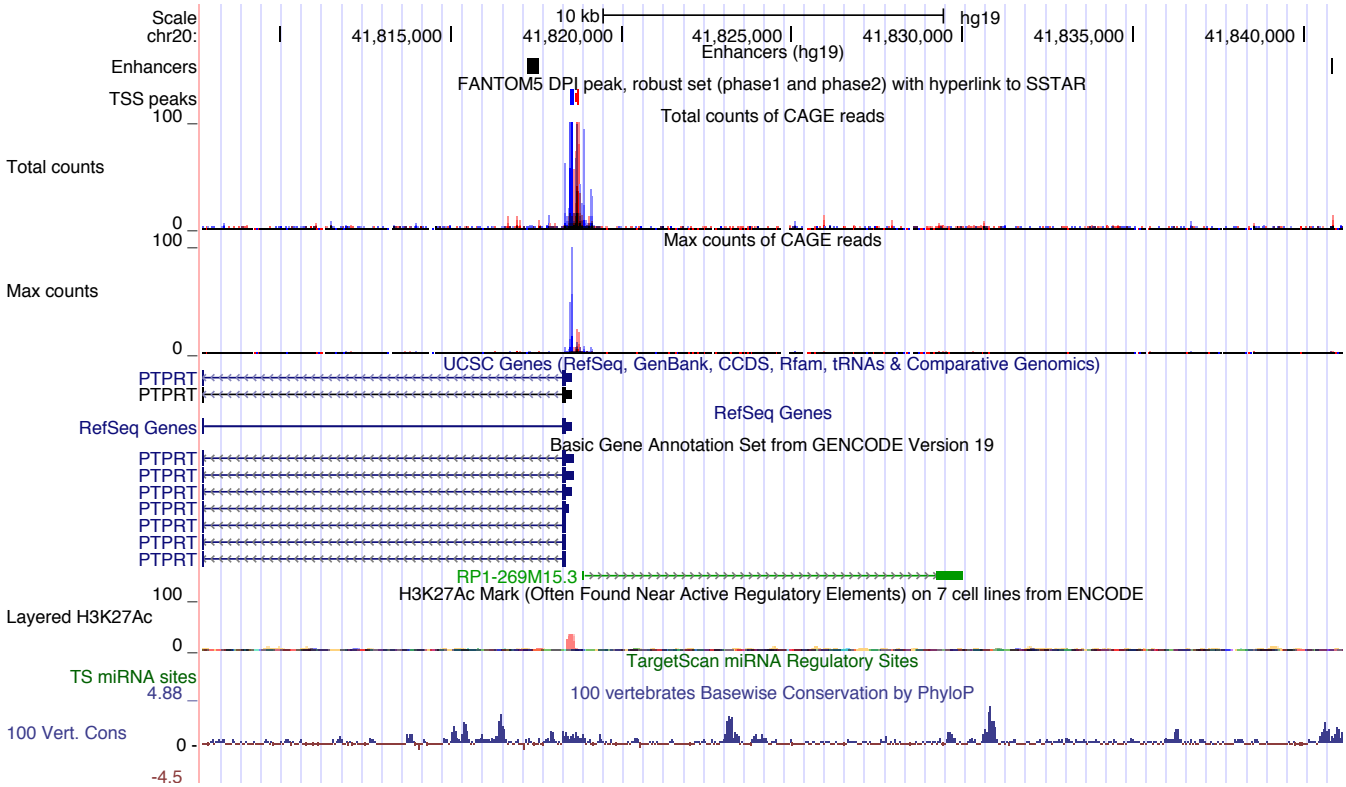

Figure s2a Functional annotation for RP1-269M15.3.

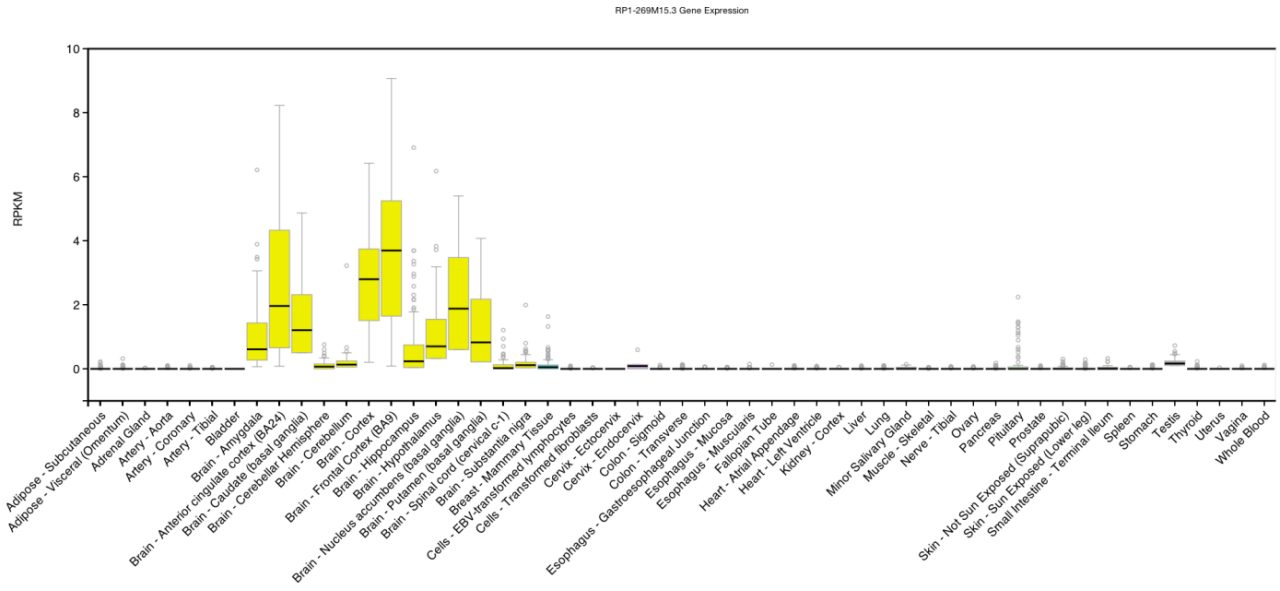

Figure s2b Gene expression pattern of RP1-269M15.3 across different tissues(downloaded from http://www.gtexportal.org/home/gene/RP1-269M15.3).

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***IN THIS ISSUE Statement**

Single-SNP-based-genome-wide analyses such as GWAS have been shown to be under powered for MDD. As an alternative approach, HRHM targets the combined effects from multiple SNPs within haplotype blocks. Using this method and a large Scottish sample we identified a 24kb region within *TOX2* gene significantly associated with MDD and this signal was further located to single SNPs with potentially functional effects by association tests. The results were replicated in independent samples.